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The characteristics of certain varieties of potato with special reference to their suitability for drying

BY W. G. BURTON, *Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge and Department of Scientific and Industrial Research*

There is considerable varietal variation in the characteristics of the potato. The characteristics of any one variety of potato may be very greatly modified by environmental conditions. Rainfall and percentage dry matter may be inversely correlated, as may rainfall and yield in localities with a medium or high precipitation. There is an apparent correlation between time of maturity, percentage dry matter, and texture when cooked, late varieties having a higher percentage dry matter and a more floury texture than early varieties. It is a fundamental requirement of potatoes for drying purposes that they should not discolour on cooking. In addition the texture of the cooked potato should be suitable for the method of drying employed, and for the type of reconstituted product desired, and the percentage dry matter should be as high as is compatible with the foregoing conditions.

INTRODUCTION

The characteristics which determine the value of any variety of potato for normal ware purposes are primarily time of maturity, yield, keeping and cooking qualities, and resistance to disease. The percentage and composition of dry matter in the tubers are rarely considered in this country, where potatoes are grown almost entirely for culinary purposes, but as will be seen later, the percentage dry matter may well be of great importance in this respect, for it can be correlated with cooking quality. All these characteristics are influenced by environmental factors such as weather, manurial treatment, and locality, but even so some varieties always mature sooner than others, some yield consistently better than others, and some are more resistant to disease, and it is, therefore, possible to classify varieties with respect to these characteristics. The characteristics of certain of the commercial varieties are given in Table 1.

TIME OF MATURITY

Potato varieties may be divided into four maturity types—first early, lifted before the middle of June; second early, lifted about a month later; early maincrop, lifted towards the end of September; and late maincrop, which are sometimes not lifted until November. The primary essential of a first early variety is that it shall catch the early markets when prices are high. Some other qualities have been sacrificed to the ability to produce a crop of reasonable size at an early date, with the result that early varieties have comparatively low yields and, usually, poorer cooking and keeping qualities. The essential requirements of maincrop varieties, on the other hand, are heaviness of yield and high cooking and keeping qualities. It must be understood that the times of maturity or lifting for the different types given above are average times and considerable variation occurs between different localities and also between different varieties within any one maturity type. For example, King Edward and Craigs Defiance mature about 3 weeks earlier than Majestic, and Bintje is earlier still, yet all are early maincrops. Similarly, Eclipse matures some 3 weeks later than Epicure, yet both are first early varieties.

The yield, cooking quality, and percentage dry matter in the tubers of the various maturity types will be dealt with later under these headings.

YIELD

A good maincrop variety should yield between 10 and 20 tons/acre, depending upon the locality and the manurial treatment. Early varieties give approximately half this yield. A high proportion of ware is essential, and although the arbitrary definition of the size of ware is varied from time to time as a means of controlling the supply, it may be said that tubers which will not pass through a 2 in. riddle are 'ware'; those that will pass through a 2 in. riddle but will not pass through a 1½ in. riddle are 'seed', and the remainder are 'chats'. A good variety should have something of the order of 80–90% of its yield as ware and not more than 1–2% as chats.

The values for 'yield' given in Table 1 are based upon growers' experience extending over many years and are comparative values only. For example, the yield of Epicure is described as 'excellent'. This is in comparison with other first early varieties and does not mean that Epicure can compete in yield with the early maincrop Majestic, the yield of which is also described as 'excellent'.

PERCENTAGE DRY MATTER

Except where otherwise stated the figures for the percentage dry matter given in Table 1 have been taken from the *Report on the Composition of Potatoes Grown in the United Kingdom* published by the Roya Society Food (War) Committee in 1919. The

Table 1

I. Immune from wart disease. S. Susceptible to wart disease. Sh. shallow, M. medium, D. deep eyes.

Numbers in parentheses refer to the number of samples upon which the dry matter figures are based

Variety	Yield	Av. dry matter %	Resistance to disease	Keeping quality	Cooking quality
First earlies					
Arran Pilot	Very good	17·8*	I. Very susceptible to most of the virus diseases. Very susceptible to dry rot	Fair	Pale lemon colour. Sh.
Di Vernon	Very good	—	I. Fairly resistant to most virus diseases. Very susceptible to dry rot	Poor	Lemon colour; waxy. Sh.
Duke of York	Excellent	—	S. Very susceptible to blight. Susceptible to most virus diseases	Poor	Very yellow; close. Sh.
Eclipse	Good	19·0	S. Not subject to blight. Effects of *Solanum* virus 14 very severe. Resistant to many of the virus diseases	Good	Waxy. Sh.
Epicure	Excellent	20·23* (2)	S. Virus diseases as Eclipse	Good	Close. D.
Ninetyfold	Excellent	20·0*	S. Virus diseases as Eclipse. Very susceptible to dry rot	Poor	Close to waxy. Sh.
Sharpe's Express	Fair	20·5†	S. Fairly resistant to blight. Resistant to most of the virus diseases. Rather susceptible to dry rot	Good	Slightly yellow; close to waxy. Sh.
Second earlies					
Ballydoon	Fairly good	—	I. Very susceptible to blight. Resistant to most of the virus diseases	Good	M. —
British Queen	Very good	22·39±0·21 (17)	S. *Solanum* virus 14 fairly severe. *S.* viruses 1 and 3, 2, 6, very severe. Rather subject to blackleg	Good	Floury. Sh.
Catriona	Very good	—	I. Very susceptible to dry rot	Fair	Lemon colour; close Sh.
King George V	Good	21·25±0·18 (7)	I.	Good	Close. Sh.
Myatt's Ashleaf	Fair	—	S. Very susceptible to blight	Fairly good	Flesh pale yellow; floury. Sh.
Early maincrops					
Ally	Very good	20·9 (1)	I. Fairly resistant to blight. Effects of *Solanum* virus 14 not severe	Good	Darkens badly; close to soapy; insipid. Sh.
Arran Banner	Excellent	22·2‡	I. Fairly resistant to blight	Good	Susceptible to hollow centre; floury. M.
Arran Consul	Very good	—	I. Fairly resistant to blight. Susceptible to, and severely affected by *Solanum* virus 14. Subject to blackleg	Very good	M. —
Arran Peak	Very good	—	I.	Good	M. —
Bintje	Good	—	S.	Good	Yellow; waxy. Sh.
Craigs Defiance	Good	—	I. Said to be resistant to *Solanum* viruses 1, 3, 4 and 5	Sprouts very early	Very white; floury. Sh.
Doon Eire	Excellent	—	I.	Good	Rather susceptible to hollow centre; floury Sh.
Doon Star	Very good	—	I. Very subject to dry rot and to some extent to gangrene	Fairly good	Floury. Sh.
Eightyfold	Good	—	S.	Poor	Waxy. Sh.
Evergood	Fair	20·83±0·17 (20)	S. Fairly resistant to blight	Good	Pale lemon colour. Sh.
Gladstone	Good	23·2‡	I. Fairly resistant to blight	Good	Very susceptible to hollow centre; floury Sh.
Great Scot§	Very good	22·63±0·16 (24)	I. More resistant to the virus diseases than any variety in cultivation	Good	Close to floury. M.
Industrie	Good	—	S. Considerable resistance to blight, *Solanum* viruses 14, 1 and 3	Good	Yellow; waxy. M.-D.
King Edward VII	Good	21·15±0·17 (47)	S. Susceptible to blight. Effects of *Solanum* virus 14 very severe. Resistant to the other virus diseases	Good	Retains colour very well; close. Sh.
Majestic	Excellent	22·7‡	I. Resistant to blight. Very susceptible to *Solanum* virus 2. Effects of *Solanum* viruses 14 and 6 fairly severe	Fairly good	Darkens; floury. Sh.
Late maincrops					
Arran Chief	Very good	23·31±0·18 (52)	S. Fairly resistant to blight. Susceptible to most of the virus diseases but resistant to *Solanum* virus 2	Good	Close to floury. M.
Arran Victory	Very good	—	I. Tubers very resistant to blight. Effects of *Solanum* virus 14 severe	Good	Floury. M.-D.
Dunbar Standard	Good	24·1‡	I. Fairly resistant to blight. Resistant to *Solanum* viruses 1 and 3. Effects of *Solanum* virus 14 severe	Good	Floury. Sh.
Golden Wonder	Fair	27·24‡ (2)	I. Susceptible to most virus diseases. Tubers fairly resistant to blight	Very good	Close to floury. Sh.
Kerr's Pink	Excellent	23·4*	I. Fairly resistant to blight. Effects of *Solanum* virus 14 very severe	Good	Floury. M-D.
Langworthy	Fair	25·3†	I. As Golden Wonder	Very good	Close to floury. Sh.
President	Good	23·49±0·40 (9)	S. Fairly resistant to blight. Effects of *Solanum* virus 14 exceptionally severe	Good	Close to floury. M.-Sh.
Up-to-Date	Excellent	21·31±0·20 (22)	S. More resistant to *Solanum* virus 14 than any other variety	Good	Close. Sh.
Up-to-Date (Irish)	Excellent	21·17±0·16 (23)			

* Based on twenty tubers from the same locality. (Determinations made at the Low Temperature Research Station.)

† Data from Johnson & Boyle (1919).

‡ Based on sixty tubers from three localities. (Determinations made at the Low Temperature Research Station.)

§ Often grown as a late second early.

figures given are average figures only. Some measure of the range of variation is given by Table 2, incorporating all available data, which gives the frequency distribution of the percentage dry matter within the vars. Evergood, Great Scot, King Edward and Up-to-Date. It will be seen that the distribution tends to symmetry about the mean value.

It may be significant that of the twenty-two values for percentage dry matter given in Table 1 the six highest are given by late maincrop varieties.

TABLE 2. *Frequency distribution of the percentage dry matter within the vars. Evergood, Great Scot, King Edward and Up-to-Date*

Dry matter %	No. of samples			
	Evergood	Great Scot	King Edward	Up-to-Date
15–16	—	—	—	—
16–17	—	—	—	1
17–18	—	—	1	2
18–19	1	—	4	3
19–20	4	—	9	9
20–21	13	2	13	16
21–22	7	6	13	24
22–23	2	9	12	15
23–24	1	8	11	9
24–25	—	7	2	5
25–26	—	—	1	—
26–27	—	—	—	—
	28	32	66	84

Of interest in this connexion are the figures given by Rathsack (1935) who carried out an extensive survey of the cooking quality of German potatoes. His average figures for the percentage dry matter of the different maturity types are as follows: first early 22·0 (six samples; three varieties); second early 22·2 (eight samples; six varieties); early maincrop 23·6 (nineteen samples; eleven varieties); late maincrop 24·3 (seven samples; six varieties, one doubtful value having been excluded).

COOKING QUALITY

The qualities discussed in this section are consistency, and colour and flavour of the tubers when cooked. The last named is too subjective to admit of any analysis beyond classification into 'strong', 'mild', or 'insipid'. The colour may be determined by two factors, the original colour of the flesh and changes brought about by cooking, such as blackening. The most common flesh colours are 'yellow' (e.g. vars. Champion, Dunbar Yeoman, Immune Ashleaf, Myatt's Ashleaf) and 'white' (e.g. Majestic, Kerr's Pink). Some varieties, such as Majestic, often blacken on cooking; others, such as King Edward VII, blacken to a less extent, but in addition to these varietal distinctions there are others due to environmental conditions. Thus, if the tuber is wilted, e.g. owing to drought or long storage, it may blacken on cooking. On the other hand, if the temperature of the environment has been high during maturation, blackening is less likely to occur (Smith *et al.* 1942). In comparison with normal potatoes, those which blacken after cooking are said to contain, in the raw state, relatively high proportions of free amino-acids (Tottingham *et al.* 1936), and, in individual tubers, to have a high cell-sap *p*H in the parts which blacken than in the parts which do not blacken (Smith *et al.* 1942). The consistency of the tuber when cooked may be considered as 'floury', 'close', 'waxy' or 'soapy', which Salaman (1926) defines as follows:

Floury. The tubers often burst spontaneously and on application of a fork break to pieces and crumble. The surface often glitters in the light.

Close. The tubers do not burst, but readily break to the fork without crumbling.

Waxy. The flesh is firm and consistent and only breaks down when definitely kneaded.

Soapy. The consistency is the same as that of the waxy flesh, but in this case the flesh appears watery and somewhat translucent.

The characteristics given can be average values only, for the cooking quality of a potato is largely dependent on the environmental conditions under which the plants are grown. Floury varieties under adverse conditions, especially prolonged exposure to air, may become close or waxy (Salaman, 1926). According to this author the cooking quality of a variety will also alter with the age of the variety, becoming more floury. An example of this is var. King Edward VII which was said to be comparatively waxy when first introduced in 1902 but is now usually close and, in some localities, even floury.

TABLE 3. *Dry matter, starch, specific gravity and consistency of ten American varieties of potato*

Variety	Dry matter %	Starch %	Consistency	Specific gravity
Green Mountain	23·75	17·74	8	1·090
Pioneer Rural	22·70	16·90	8	1·088
Houma	21·91	16·72	5	1·084
Bedago	21·37	15·77	6·25	1·080
Katahdin	21·48	15·95	5·5	1·080
Warba	20·67	15·49	5·4	1·077
Cobbler	21·05	15·38	5·5	1·077
Pontiac	20·14	14·56	5·3	1·073
Chippewa	18·34	13·30	5·1	1·070
Earlaine	19·23	13·91	2·8	1·070

The data in Table 1 suggest that there may be direct correlation between mealiness and percentage dry matter, varieties with a high percentage dry matter being more floury. This is further illustrated by the data in Table 3 (Nash, 1941), giving the percentage dry weight, percentage starch, specific gravities and comparative mealiness of ten American varieties. The table provides a good illustration of the specific gravity method of determining dry matter and probable cooking quality. The values

given are averages for four centres. A marking of 10 for consistency indicates great mealiness.

The above correlation is more clearly shown within the varieties (Table 4). This will be mentioned later when the influence of locality is discussed.

A further subdivision should be introduced in Salaman's classification of consistency, in that floury potatoes may be divided into two classes: dry and glutinous. Maincrop potatoes with a high dry matter will usually fall into the first class, whereas first early potatoes which have grown in such a habitat that they have a high dry matter usually fall into the second class. It seems probable that whilst the liability to break down on cooking depends upon the percentage of starch present, the dryness or otherwise of the mash produced may depend upon some such factor as the strength of the cell walls.

Resistance to, and effects of, disease

Disease resistance may be of importance if potatoes are grown adjacent to diseased crops or on land from which diseased crops have previously been taken. Only in the case of 'wart disease' (*Synchytrium endobioticum*) can varieties be divided sharply into immune and susceptible classes (N.I.A.B. 1933), the onset of the disease being avoided by growing only immune varieties in localities where it is prevalent. With most diseases there is no complete immunity, but there is a varietal difference in the severity of the attack. Disease may affect yield, percentage dry matter, and keeping and cooking qualities. Reliable information is lacking on the second and last of these, although it may be a relevant fact that *Solanum* virus 14 reduces the starch present in the tuber expressed as a percentage of total dry weight (Barton-Wright & M'Bain, 1932). The effects on yield are very marked, for example severe attack by *Solanum* virus 14 has been observed to reduce the yield of var. Arran Victory by 54·6%, and that of var. President by 91·3% (Barton-Wright & M'Bain, 1932), but it must be remembered that there are marked varietal differences both in susceptibility to attack and in the severity of the symptoms (Table 1, part of the information regarding disease from Davidson, 1937). Attack by the complex of *Solanum* viruses 1 and 3 also leads to a considerable reduction in yield. Wart disease may practically destroy the whole crop.

The more serious diseases leading to rotting of the tuber during storage are blackleg (*Bacterium phytophthorum*), blight (*Phytophthora infestans*), dry rot (*Fusarium caeruleum*), watery wound rot (*Pythium ultimum*), gangrene and *Phoma* rot (*Phoma foveata*). Susceptibility to these diseases exhibits considerable varietal variation, e.g. Arran Pilot, Ninetyfold, Doon Star and Catriona are very susceptible to dry rot, and King Edward VII to blight. It is said that susceptibility changes with the age of the variety, increased age apparently leading to less liability to attack by blackleg and greater susceptibility to blight (e.g. King Edward VII was said to be practically immune to this disease when first introduced).

The effect of environmental conditions

The above varietal characteristics are much influenced by environmental factors which are discussed below.

Table 4. *Dry matter, starch and consistency in different localities*

Variety ...	Green Mountain			Houma			Cobbler			Chippewa		
Locality	Dry matter %	Consistency	Starch %	Dry matter %	Consistency	Starch %	Dry matter %	Consistency	Starch %	Dry matter %	Consistency	Starch %
Tompkins	25·80	10	19·26	22·48	4	16·79	22·00	9	16·13	19·01	2	13·44
Steuben	25·22	10	19·26	23·67	6	18·36	21·07	6	16·05	20·44	6·5	14·74
Suffolk	22·53	6	16·45	21·78	3	16·17	20·60	4	14·96	17·63	2	13·13
Wayne	21·44	6	15·97	19·69	2	15·54	20·52	3	14·39	16·28	1	11·88

(*a*) *The effect of weather conditions.* The weather may influence the value of a potato crop both directly by its effects on yield, percentage dry matter and cooking quality, and indirectly by its effects on the spread of disease. For instance, sultry humid weather favours the spread of *Phytophthora infestans*, and cold moist winds tend to keep virus-transmitting aphides off the wing. From the point of view of drying the produce, the direct effects are of more interest. Brandreth (1937) gave detailed weather data in addition to the results of cooking tests over 2 years, but no conclusion can be drawn from his figures as to the effect of weather conditions on the cooking quality. Such effects will be discussed later in the section on the effect of locality. If a drought in the middle of the growing season is followed by heavy rain and second growth, cavities may develop in the centres of tubers of varieties (e.g. Gladstone, Arran Banner) which are prone to this complaint. King Edward VII, the tubers of which are very similar to those of Gladstone, scarcely ever has hollow tubers. Majestic tends sometimes to hollowness.

The effects of weather on yield are very complex and such data as exist are difficult of interpretation.

Extensive tables on the effect of weather condi-

tions on yield have been published by the United States Departments of Agriculture and Commerce (Bean, 1942). The rainfall of each State is given in the form of deviations from the mean for each month, this being also given. Numerous errors occur in the rainfall tables. For example, the sum of the published deviations from the main rainfall for the period 1873–1939 is +0·70, this error being introduced by the following errors in the sums of the published monthly deviations (Jan.–Dec. in order): +0·31, −0·33, −0·01, −0·10, −0·74, +0·18, +1·90, −0·28, −0·18, +0·15, −0·26, +0·06.

Even given accurate tables of such a type, their usefulness is limited by several factors. Methods of cultivation, and the varieties grown, have improved to a considerable extent in the long periods covered by the tables (over 70 years in some cases). The yield figures thus exhibit an upward trend which may entirely obscure the effects of weather. The State of California affords a good example of this, the average yield over the period 1867–76 being 90 bushels/acre, whilst over the period 1930–9, the average yield was 243·6 bushels/acre. An additional factor is that the mean rainfall in any State does not necessarily represent the mean rainfall in the potato-growing areas in that State, and is in any case very much a function of the distribution of meteorological stations. The American tables also give deviations from the mean temperatures for each month, but do not give hours of sunlight.

The effect of weather depends upon so many factors that it is difficult, without more data than are at our disposal, to evaluate the effect of any one factor. The only satisfactory data would be those determined on one variety of potato grown under standard cultural conditions on the same type of soil in the same district over a considerable number of years.

Bearing the above difficulties in mind it seems best to treat the American data in groups of 10 years dealing only with small States with climates reasonably uniform throughout the State. The relevant data for Rhode Island for the period 1930–9 are given in Table 5. The yield per acre may be inversely correlated with rainfall,† the correlation coefficient being −0·786 and the correlation being significant at the 2% level.

Some data relating to the variety Ally grown at Rothamsted Experimental Station, that show the effect of rainfall on percentage dry matter,* are given in Table 6.

* Dry-matter figures from a private communication from Sir E. J. Russell to the Superintendent, Low Temperature Research Station, 24 July 1941. The author is indebted to Dr W. G. Ogg for permission to publish them.

† The average annual rainfall in Rhode Island is about 44 in. evenly distributed thoughout the year. In States with a much lower rainfall (e.g. Wyoming, c. 14 in.) the inverse correlation is replaced by a direct correlation.

Dry matter and rainfall are inversely correlated, the correlation coefficient being −0·727, and the inverse correlation being significant at the 2% level.

No correlation is shown between hours of sunlight and percentage dry matter, but whether or not any exists could only be determined by the examination of data over a longer period than the 6 years for which figures are available.

TABLE 5

Year	Rainfall (May–Sept. incl.) in.	Yield bushels/acre
1930	12·25	195
1931	20·87	150
1932	18·58	160
1933	21·65	180
1934	15·01	185
1935	19·81	175
1936	15·71	180
1937	14·73	195
1938	22·59	160
1939	14·82	190

TABLE 6. *The influence of weather conditions on percentage dry matter*

Year	Rainfall (May–Sept. incl.) in.	Dry matter %	Sunlight (May–Sept. incl.) hr.
1930	11·52	23·2	—
1931	12·77	23·4	—
1932	11·01	22·7	—
1933	6·70	27·0	—
1934	8·10	24·6	989·2
1935	11·51	23·6	1029·0
1936	14·58	23·5	758·2
1937	11·26	24·3	803·4
1938	7·58	24·7	778·0
1939	9·99	23·3	815·5

A further illustration is provided by trials carried out at the Albert Agricultural College, Glasnevin, Co. Dublin (Table 7) (yield and percentage starch figures from Drew & Deasy, 1939).

It is clear that rainfall and percentage starch in Table 7 are inversely correlated. No correlation is apparent between rainfall and yield, but there is a direct correlation between yield and hours of sunlight divided by rainfall. Whether this is significant could only be determined by an examination of results over a large number of years.

(*b*) *The effect of manurial treatment.* The only reliable data available relate to trials at the Rothamsted Experimental Station and are given in Table 8 (Gilbert, 1888).

There is a direct correlation between percentage dry matter and infertility in the above series, but it should be noted that percentage dry matter and amount of dry matter per acre are in no way comparable, the maximum reduction from 28·1 to

25·6% in the percentage dry matter shown in Table 8 being accompanied by a 235% increase in yield.

Drew & Deasy (1942) found that the substitution of potassium sulphate for potassium chloride in artificial manures increased the percentage of starch in the tubers, but in some cases decreased the yield. The former effect was the more marked, however, the net result being an increase in the yield of starch per acre.

(*c*) *The effect of locality.* The locality where any variety of potato is grown may modify its characteristics, probably mainly by reason of differences in rainfall and type of soil. The most detailed work on the influence of locality on cooking quality available for tubers grown in this country was done by the National Institute of Agricultural Botany in collaboration with the research department of Messrs J. Lyons and Co. Ltd. (Parker *et al.* 1934; Brandreth, 1937). They concluded that the influence of soil on potato quality lay in its effect on consistency when boiled, Dunbar red sandstone giving the best, and black fen and sandy loam the worst consistency. Different types of soil produced no difference in colour or flavour. The ideal consistency was taken to be 'soft and floury, retaining its shape when handled, but easily mashed'.

In experiments carried out in four different regions of New York State (Nash, 1941), the average texture of ten varieties of potato ranged from a rating of 7·5 for Tompkins County to 2·8 for Wayne County, a range of 1 to 10 being used to describe variations from 'extremely soggy' to 'most mealy'. Similarly, the colour half an hour after boiling varied from 7·3 for Steuben County to 10·0 for Wayne, the range 1–10 illustrating gradations from 'extremely black' to 'white'. Cobb (1935) stated that temperature is the most important environmental factor influencing the consistency of the cooked potato, but rainfall may be more important if, as has been suggested above, mealiness may be correlated with percentage dry matter, and the latter inversely correlated with rainfall. It has also been suggested that low temperatures and lack of sufficient sunlight favour the occurrence of blackening in the cooked product (Nash, 1941; Smith *et al.* 1942). Blackening has also been observed in cooked tubers that had been wilted by drought in the middle of the growing season, and in tubers that had wilted during storage.

TABLE 7. *Results of trials at the Albert Agricultural College, Glasnevin, Co. Dublin*

Yields are given in tons/acre. Rainfall and sunlight figures are for the months June, July and August only. Percentage starch was calculated from the density.

			Arran Banner		Arran Consul		Kerr's Pink		Up-to-Date	
Year	Rainfall in.	Daily average sunshine hr.	Yield	Starch %	Yield	Starch %	Yield	Starch %	Yield	Starch %
1935	6·01	6·01	20·05	14·41	16·91	15·31	18·67	15·67	19·60	15·56
1936	9·47	5·12	15·96	12·91	14·61	14·14	12·99	14·46	12·97	13·98
1937	5·89	4·42	16·70	13·49	16·50	16·12	15·50	16·72	12·70	15·60

The effect of locality on yield may be illustrated by yield trials carried out on Arran Cairn in 1935 in Eire (Drew & Deasy, 1939). The average yield from sixteen centres in Co. Cork was 14·62 tons/acre; that from ten centres in Co. Tipperary was 16·73 tons/acre. More noticeable, but less significant, was the fact that the yield from one centre in Co. Meath was 9·54 tons/acre and the average yield from two centres in Co. Longford was 20·55 tons/acre. The soil in Co. Cork might be said on an average to be a light loam, that of Co. Tipperary a clay.

TABLE 8. *Effect of manurial treatment. Average results over the* 12 *years* 1876–87. *Vars. The Rock* (1876–9) *and Champion* (1880–7)

	Yield tons/acre	Diseased tubers %	Dry matter %	Specific gravity
Unmanured	2·0	3·15	28·1	1·118
Dung (average 6 years) (1876–81)	5·2	5·54	25·8	1·106
Superphosphate	3·7	3·66	26·8	1·115
Mixed mineral manure	3·8	3·45	26·5	1·114
Ammonium salts alone	2·3	4·06	26·2	1·110
Nitrate of soda alone	2·6	4·93	26·5	1·112
Ammonium salts + mixed mineral manure	6·7	6·26	25·6	1·104
Nitrate of soda + mixed mineral manure	6·7	7·00	25·8	1·108

The percentage dry matter of all varieties of potato must be influenced considerably by the environment. This is illustrated by Table 9 where are given the frequency distributions of percentage dry matter in the 1917 crop of vars. Arran Chief, British Queen, Great Scot and King Edward VII (calculated from the individual values given in the *Royal Society Food (War) Committee Report* 1919) for the regions east,

west, north and south. The line of division between east and west has been taken as 2° W. and between north and south as the latitude of Nottingham. The mean value for the west is 21·85%, the variance of the mean being 0·0545, and for the east is 22·81%, the variance of the mean being 0·0445. The difference between the means is thus 0·96%, and its standard error ±0·315, the corresponding value of '*t*' being 3·05. There is thus a difference, significant at the 1% level, between the average dry matter of tubers grown in the east and those grown in the west, the former having a higher value. No such difference exists between north (mean value 22·46%) and south (mean value 22·37%), the difference between the means being 0·09 and the standard error of this difference ±0·299.

TABLE 9. *Frequency distribution of percentage dry matter of the vars. Arran Chief, British Queen, Great Scot and King Edward VII*

Dry matter %	East	West	North	South
15–16	—	—	—	—
16–17	—	—	—	—
17–18	—	2	—	2
18–19	2	2	—	4
19–20	6	4	3	7
20–21	7	10	6	11
21–22	9	14	9	14
22–23	15	14	15	14
23–24	21	5	9	17
24–25	10	7	6	11
25–26	7	2	1	8
26–27	3	—	1	2
27–28	—	—	—	—
	80	60	50	90

CHOICE OF POTATOES FOR DRYING

The characteristics which would appear to determine suitability for drying are primarily cooking quality and percentage dry matter. Obviously a potato which retains its colour well on cooking is necessary, but this is probably determined by environmental conditions at least as much as by variety. Little systematic work has been done on the blackening of different varieties of potato in this country, but var. King Edward VII is known to be not very susceptible to this defect, whilst Majestic is rather prone to it. The importance of consistency depends upon the drying process employed and the type of reconstituted product desired. In processes involving the drying of mashed potato or potato suspensions a floury texture is to be preferred; on the other hand, if cooked strips or other comparatively large pieces are to be dried, a waxy texture may be preferable. To reduce trimming losses the eyes should be shallow and the tubers regular in shape.

Waxy potatoes do not mash readily, so the use of floury maincrop varieties of potato is suggested for drying when mash is required after reconstitution, and first early varieties if the reconstituted product is to be pieces of firm texture. If strips are to be dried in a partially cooked condition the principal requirements are that the strips shall retain a reasonably firm consistency when adequately blanched, that they shall reconstitute without breaking down completely, but nevertheless mash readily. Both very floury and waxy potatoes are unsuitable under these conditions, and it seems probable that varieties such as Catriona, King Edward VII or Up-to-Date, which have a close texture when cooked, would be the most suitable for drying by this process. It is of interest in this respect that in America, where potatoes are principally dried in the form of partially cooked strips, emphasis is laid upon the necessity for using varieties that do not blacken after cooking and that are mealy in texture. Idaho Russet, Oregon Gem, Klamath Russet, Burbank, Irish Cobbler, Early Ohio, Chippewa and Bliss Triumph have been used with success, but these vary in consistency, Irish Cobbler for example being mealy (Clark *et al.* 1940) whilst Chippewa has a tendency to be soggy (Nash, 1941).

If percentage dry matter is taken into consideration, and since it determines yield it should be, it seems probable that late maincrop varieties would be the most valuable. The locality where grown is also of importance in this respect.

In addition to the above considerations, however, the availability of the different varieties must be borne in mind. In England and Wales the early maincrop vars. Majestic, King Edward, Doon Star and Arran Banner are the most widely grown, comprising some 80% of the maincrop acreage; in Scotland, Kerr's Pink occupies about 30% of the maincrop acreage, and in Northern Ireland Arran Victory and Kerr's Pink comprise over 74% of the total acreage; thus, in England and Wales early maincrop varieties with a comparatively low percentage dry matter are much more widely grown than late maincrops. The most popular variety is the heavy-cropping Majestic which is of only fair cooking and keeping quality and tends to blacken when cooked, which fault may make it unsuitable for drying. Next in order of popularity comes the high-quality and comparatively low-yielding King Edward VII, which also possesses the disadvantage of being susceptible to wart disease. This variety would seem very suitable for drying by any process, but has a rather low dry-matter content.

In Scotland and Northern Ireland, on the other hand, heavy-yielding and high-quality late maincrop varieties are most popular, and these would seem to be eminently suitable for any process involving drying cooked mashed potato or potato suspensions. In the case of Arran Victory the colour of the product might be adversely affected by the purple pigment in the flesh of the tuber below the eyes.

As mentioned above, if comparatively large pieces

of potato are to be dried, and if it is desired that the reconstituted product contain separate firm pieces, first early varieties would seem the most suitable. These occupy some 13% of the total potato acreage in England and Wales, but very much less in Northern Ireland and Scotland. The principal variety is Arran Pilot, followed by Eclipse, Ninety-fold and Epicure, these making up over 80% of the first earlies grown.

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REFERENCES

Barton-Wright, E. & M'Bain, A. (1932). Studies in the physiology of the virus diseases of the potato: a comparison of the carbohydrate metabolism of normal with that of leaf-roll potatoes. *Trans. roy. Soc. Edinb.* **57**, pt. 2, 309.

Bean, L. H. (1942). Crop yields and weather. *Misc. Publ. U.S. Dep. Agric.* no. 471.

Brandreth, B. (1937). Potato quality trials, 1935 and 1936. *J. nat. Inst. agric. Bot.* **4**, 193.

Clark, C. F., Lombard, P. M. & Whiteman, E. F. (1940). Cooking quality of the potato as measured by specific gravity. *Amer. Potato J.* **17**, 38.

Cobb, J. S. (1935). A study of culinary quality in white potatoes. *Amer. Potato J.* **12**, 335.

Davidson, W. D. (1937). *Potato Growing for Seed Purposes.* Dublin Stationery Office.

Drew, J. P. & Deasy, D. (1939). Potato growing in Ireland with particular reference to production for industrial purposes. *J. Dep. Agric. Eire*, **36**, 205.

—— —— (1942). The influence of manures on the percentage of starch of potatoes and on the total yield of starch. *J. Dep. Agric. Eire*, **39**, 35.

Gilbert, J. H. (1888). Results of experiments at Rothamsted, on the growth of potatoes, for twelve years in succession on the same land. Reprinted (1893) as Paper 13, *Roth. Mem. agric. Chem.* (Sci.), **6**.

Johnson, T. & Boyle, C. (1919). Observations on the industrial and nutritive value of the potato in Ireland. *J. Dep. Agric. Ire.* **19**, 416.

Nash, L. B. (1941). Relation of variety and environmental conditions to partial composition and cooking qualities. *Amer. Potato J.* **18**, 91.

National Institute of Agricultural Botany (1933). *Varieties of Potatoes with their Synonyms Immune from and Susceptible to Wart Disease.*

Parker, W. H., Salaman, R. N. & Brandreth, B. J. (1934). Cooking quality in potatoes. *J. nat. Inst. agric. Bot.* **3**, 408.

Rathsack, K. (1935). *Der Speisewert der Kartoffel.* Verlagsgesellschaft für Ackerbau m.b.H.

Royal Society Food (War) Committee (1919). *Report on the Composition of Potatoes Grown in the United Kingdom.*

Salaman, R. N. (1926). *Potato Varieties.* Cambridge.

Smith, O., Nash, L. B. & Dittman, A. L. (1942). Potato quality. VI. Relation of temperature and other factors to blackening of boiled potatoes. *Amer. Potato J.* **19**, 229.

Tottingham, W. E., Nagy, R. & Ross, A. F. (1936). The problem of causes of blackening in cooked potatoes. *Amer. Potato J.* **13**, 297.

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Influence of certain soil factors on chocolate spot of beans

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In the summer of 1941 chocolate spot of beans was widespread in the South-Eastern Agricultural Province and caused much damage to the crop. Soil samples were collected from forty-nine affected fields on a variety of soil types, and the relation between the severity of attack by chocolate spot and the texture, *p*H, available potassium and available phosphorus determined. Severity of attack was classified into three grades: 'slight', in which only spotting of the foliage occurred; 'moderate', in which death of leaves and blossoms on the three or four lowest nodes occurred; and 'severe', in which there was generally a total loss of crop. No significant relation between severity of attack and soil texture, *p*H or available potassium was found. A highly significant relation was found to exist between the severity of attack and the amount of available phosphorus in the soil as determined by the method of soil analysis used in this Province. Damage by chocolate spot was generally slight on soils containing 'medium to medium high' or higher amounts of available phosphorus, and generally severe on soils containing low amounts of available phosphorus.

Introduction

Various writers have expressed the opinion that soil conditions influence the occurrence of chocolate spot in beans. Beaumont (1932–3), Cowie (1936), Scott-Watson (1936), Glynne (1936), and Wilson (1937*a*, *b*) produced evidence of an inverse relation between the severity of attack and the amount of available potassium in the soil. Beaumont (1933) stated that the soils where the disease occurred were deficient in potash either in the actual amount of available potash or in the amount relative to the available phosphates. Wilson (1937*a*) concluded that any factor tending to weaken the plant, e.g. acidity, waterlogging and deficiency of available potassium or phosphorus, appears to render it more vulnerable.

In the summer of 1941, chocolate spot was widespread and caused considerable damage to beans in the South-Eastern Province. An investigation by Ware & Glasscock (1941) of the influence of cultural factors and weather on the incidence of the disease showed that attack was prevalent during moist weather conditions, such as mist, drizzle or light rain, between mid-May and mid-June, and that it ceased when the weather became hot and dry on 13 June. About 100 fields of beans were examined when dry weather had stopped the disease, and the extent of attack was classified into three grades: slight, moderate and severe. In view of the conclusions reached by others regarding the influence of soil factors, it was decided to extend the investigation to see whether severity of attack could be related not only to weather conditions but also to those soil factors normally determined in routine advisory work, namely, texture, *p*H, available potassium and available phosphorus. In the collection of soil samples it was necessary to enlist the help of the farmers whose fields had been examined. Detailed instructions on soil sampling were sent to each one and through their courtesy forty-nine samples of soil taken at 0–6 in. were eventually received. The fields were well distributed over the area of the Province and, in view of their random selection, were probably representative of the chief types of soil occurring in the Province.

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Methods

The three grades into which damage by chocolate spot was classified were as follows:

A. *Slight*, in which damage was confined largely to spotting of the foliage.

B. *Moderate*, in which death of leaves and blossoms on the three or four lowest nodes occurred.

C. *Severe*, in which damage was of the 'aggressive' type described by Wilson (1937 *a*, *b*) and in which there was generally a total loss of crop.

It should perhaps be pointed out that each grade, particularly the 'moderate' grade, covers a broad range of damage and that it describes the general condition in a field or part of a field.

The methods used for determining the soil factors were as follows:

Texture, by feel of the moist soil between the thumb and fingers. The soil is placed in one of twenty-one textural groups which are those of the U.S. Bureau of Chemistry and Soils, together with the addition of the group 'silty loam' between 'loam' and 'silt loam'. Either a number (1–21) or a description may be used to define texture.

*p*H, by the quinhydrone electrode using a soil/water ratio of 1/2·5.

Available potassium and phosphorus, by the modified Morgan method described by Tinsley & Pizer (1940 *a*, *b*). Results are expressed as parts per million (p.p.m.) of potassium or phosphorus in the soil extract.

Influence of soil texture

The common textural classes of the soils of the south-east of England are: very fine sandy loam, loam, silty loam and silt loam. Very light soils occur in certain areas, but very heavy soils are uncommon. The south-east is therefore not a very suitable area for examining the relation between soil texture and the incidence of chocolate spot. As might be expected the soils examined were mainly of medium texture—loams, silty loams and silt loams. In each grade of damage the mean value for texture was 1·4 or silty loam with standard deviation 1·5 or less. The heaviest soils were clay loams (heavy loams), and on them damage by chocolate spot was generally slight. The lightest soils were fine sandy loams (light loams), and on them damage was generally severe. Nothing more definite than this indication that texture might be related to the occurrence of chocolate spot was found.

Influence of *p*H

The data on *p*H are summarized in Table 1. The mean values for each grade lie between 6·0 and 7·0, and the differences between them are not significant. The range of values was 5·8–7·5 in grade A, 4·8–7·5 in grade B, and 5·3–7·5 in grade C. Apart from any consideration of disease incidence, it is interesting to note that in a soil with *p*H as low as 4·8 the grower reported a moderate crop.

Influence of available potassium and available phosphorus

The figures obtained for available potassium are directly related to what is known as the exchangeable potassium which is generally considered to provide the greater part of the potassium taken up by plants. 10 p.p.m. of potassium in the soil extract are approximately equivalent to 50 p.p.m. of exchangeable potassium oxide in the air-dry soil.

The method used for determining available phosphorus provides a measure of the rate of availability of soil phosphates and thus differs from most other methods in use.

The data on available potassium and available phosphorus are summarized in Table 1. The mean values decrease steadily as the severity of attack by chocolate spot increases, indicating a possible relation between attack by chocolate spot and the amounts of available potassium and available phosphorus in the soil.

The range of values for available potassium was 20–70 p.p.m. of potassium (low to extra high) in grade A, 10–70 p.p.m. (very low to extra high) in grade B, and 10–50 p.p.m. (very low to high in grade C). First inspection of the figures suggested that no significant relation between available potassium and chocolate spot existed. This was found to be the case. Examination of the figures by the method of analysis of variance showed that the gradual fall in the mean values for available potassium from grade A to grade C was probably largely due to a significant positive correlation of available potassium with available phosphorus.

The range of values for available phosphorus was 1·1–4·5 p.p.m. of phosphorus (low to high minus) in grade A, 0·75–3·0 p.p.m. (low to medium high) in grade B, and 0·75–1·5 p.p.m. (low to medium) in grade C. Examination of the figures by the method of analysis of variance and applying the F and t tests showed that the grade means (Table 1) differed significantly from each other. The probability that fields in grade A differed significantly as regards available phosphate from the fields in grade C was less than 0·01 and from the fields in grade B was between 0·05 and 0·01. The probability that the fields in grade B differed from those in grade C as regards available phosphate was between 0·05 and 0·01. The probability of significant difference is thus very high between the slight and severe grades of damage, but less high between the moderate and slight grades or the moderate and severe grades. This is to be expected owing to the lower degree of certainty of placing damage by chocolate spot in grade B as compared with grades A or C.

There is thus a very definite relation between the severity of attack by chocolate spot and the availability of phosphorus in the soil as measured by the particular method of analysis used. On the soils examined, where available phosphorus was greater than 2 p.p.m. damage by chocolate spot was generally slight, and where it was less than 1 p.p.m. damage was generally severe. For arable crops these values of available phosphorus are medium to medium high and low respectively. Since 75 % of the thousands of soil samples analysed in the South-Eastern Province contain medium or smaller amounts of available phosphorus, phosphates would appear to be generally necessary to reduce damage by chocolate spot in bean crops in seasons in which the disease is active, assuming that the greater resistance shown

Table 1

Grade of damage	No. of fields	*p*H		Available potassium		Available phosphorus	
		Mean value	Standard deviation	Mean value p.p.m.	Standard deviation	Mean value p.p.m.	Standard deviation
A. Slight	8	6·71	0·69	39·38	23·05	2·25	1·116
B. Moderate	27	6·76	0·76	27·96	13·68	1·583	0·723
C. Severe	14	6·19	0·74	23·57	10·67	1·073	0·324

to the disease is in fact due to available phosphate and not to some other factor that accompanies it.

Whatever the mechanism may be, it appears that in an epidemic year bean plants suffered little damage by chocolate spot on soils containing medium to medium high amounts of available phosphorus, and serious damage amounting to total loss of crop on soils containing low amounts of available phosphorus.

Possible reasons for the relationship suggested above are as follows. Readily available phosphate has a number of effects on the soil and on the plant. In the soil it stimulates biological activity and may thus increase or decrease the supply of plant foods. It promotes growth of the leaves and roots of seedlings and causes an increase in the fibrous root system. In beans this increase in the fibrous root system might be followed by an increase in the number of root nodules, and the greater and more efficient root system would enable the plant to obtain from the soil a larger supply of essential nutrients. In the plant, phosphorus is required to regulate the nitrogen metabolism. It counteracts the effects of nitrogen and tends to produce a harder growth.

Variations in attack in the same field

In many fields attack by chocolate spot was not uniformly severe, and in certain fields there were 'islands' or areas where attack was much less severe. Where marked differences in severity of attack occurred it was usually possible to establish from the past history of the field, or by soil analysis, that in the areas where attack was severe the soil was in a less fertile condition than where attack was slight. For instance, in one field the crop was generally poor and severely attacked by chocolate spot, but in one small area the beans were excellent and only slightly attacked. Analysis of the soil showed extra high available potassium and medium high available phosphorus in the good area and low amounts of available potassium and phosphorus elsewhere. At first sight this seemed a straightforward instance of the effects of potassium and phosphorus, but it was then found that the good area had been a site for hay ricks round which sheep had collected, and thus differed in many ways from the rest of the field. In another field the previous crop had been tares and part had been eaten off by sheep and part cut and carted off. Attack by chocolate spot was less severe where the tares had been fed off. Analysis showed that the field was in poor condition. Available phosphorus was low all over and available potassium low in the better area and very low in the poorer area. The effects of grazing or cutting the previous crop seemed therefore to be the chief cause of the difference between the two areas. In another field, attack was moderate where bracken had recently grown, and severe elsewhere. As is usually found, the bracken area was extra high in available potassium, but the rest of the field was nearly as well supplied with potassium. All the field was low in available phosphorus. This instance and the ones previously mentioned have this feature in common, that where organic matter was more abundant in the soil or had been recently added, damage by chocolate spot was slight or less serious than elsewhere. The effects of organic matter in the soil are numerous, but the structure of the soil is usually improved and made much better for root development and hence the plant is able to develop a better root system and increase its capacity to obtain nutrients from the soil. This explanation is in line with that previously advanced for the effect of phosphate.

REFERENCES

Beaumont, A. (1932). *Rep. Seale-Hayne Agric. Coll.* 1930–1, p. 19.

—— (1933). *Rep. Seale-Hayne Agric. Coll.* 1931–2, p. 21.

Cowie, G. A. (1936). Chocolate spot in beans. *Fertil. Feed. St. J.* **21**, 182.

Glynne, M. D. (1936). Fungus and other diseases at Rothamsted and Woburn, 1935. *Roth. Exp. Sta. Rep.* 1935, p. 84.

Scott-Watson, J. A. (1936). Notes on manuring. *J. Minist. Agric.* **43**, 178.

Tinsley, J. & Pizer, N. H. (1940*a*). An examination of the Morgan rapid method of soil testing. Part I. The analytical procedures for phosphorus, potassium and calcium. *J. Soc. chem. Ind., Lond.*, **59**, 206.

Tinsley, J. & Pizer, N. H. (1940*b*). An examination of the Morgan rapid method for soil testing. Part II. Application to non-calcareous soils. *J. Soc. chem. Ind., Lond.*, **59**, 265.

Ware, W. M. & Glasscock, H. H. (1941). Chocolate spot in beans in 1941. *J. Minist. Agric.* **48**, 91.

Wilson, A. R. (1937*a*). The chocolate spot of broad and field beans. *J. Minist. Agric.* **43**, 1047.

—— (1937*b*). The chocolate spot disease of beans (*Vicia Faba* L.), caused by *Botrytis cinerea* Pers. *Ann. appl. Biol.* **24**, 258.

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Occurrence of *Ophiobolus graminis* var. *Avenae* on wheat crops in the field

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Ophiobolus graminis var. *Avenae* has been found on oat crops in ten counties in Scotland. It has also been shown to attack wheat crops in the same districts.

The widespread occurrence of *Ophiobolus graminis* var. *Avenae* on oats in the west and north of Scotland was demonstrated by a crop survey carried out in August 1942, a report of which has already been published (Garrett & Dennis, 1943). In this report it was pointed out that in the only instance where wheat and oat crops growing side by side each showed symptoms of take-all, the wheat was found to be infected by *O. graminis* proper whereas the oat straws bore *O. graminis* var. *Avenae*. In view of the demonstration (Turner, 1940) that the latter fungus could attack wheat in sand cultures it was suggested that a special search should be made for it on that host in the field in 1943.

With a view to confirming and extending the observations of the previous year's survey 104 oat crops were examined in August 1943, mainly in south-west Scotland, ninety of which showed varying amounts of take-all. Only in twenty-six fields, however, was there sufficient disease to cause appreciable loss of crop, usually in cases where a third successive oat crop had been grown or on land which had been waterlogged during the previous winter or early spring and had consequently lost much of its fertility by leaching. This disease has now been recorded in the following counties: Banffshire, Aberdeenshire, Kincardineshire, Perthshire, Fife, Peeblesshire, Dumfriesshire, Kirkcudbrightshire, Wigtownshire, and Ayrshire and is no doubt present in oats throughout the wetter and higher-lying districts.

Opportunity was taken to examine as many wheat crops as possible in the area visited, special attention being paid to wheat following oats. Take-all samples were secured from fifteen wheat crops, seven of which had followed oats, and were tested for presence of *O. graminis* var. *Avenae* by the method of Garrett (1938). This consists of inserting an oat grain within the lumen of the infected straw, planting in sand culture and examining the seedling for take-all lesions. Six of the samples bore mature perithecia from which ascospores were ejected and measured as a check on the biological test. The results are summarized in Table 1, in which is included for comparison data for a typical infection on oat straw after old lea and the result of inoculation of 100 oats with an agar culture derived from ascospores ejected from perithecia on wheat sample 89.

The oats used in the test were an unnamed sample of potato type from the 1942 crop, supplied by the Department of Agriculture's Seed Testing Station and, like almost all Scottish oats (Dennis, 1943) were infected with *Helminthosporium Avenae* and *Fusarium* spp., in this case to the extent of about 10 and 25 % respectively. It was considered undesirable to treat the seed with an organo-mercury seed disinfectant before insertion in the straw, consequently interpretation of the results is complicated by the presence of *Fusarium* footrot. Leaf stripe, though present to a small extent, differs so much in its effect on the plant from take-all and other footrots that it can be neglected for the present purpose. While there was no difficulty in distinguishing between take-all and *Fusarium* footrot, it might be argued that oat seedlings affected by the latter may be more susceptible than normal plants to attack by *Ophiobolus graminis* proper. No support for such a hypothesis can be found in the literature, however, and in the case of samples 23, 29, 80, 83, 85, 120, 122 and the culture from sample 89 the proportion of oat seedlings showing take-all lesions is far higher than could be explained in this way and is of as high an order as with sample 10, which consisted of naturally infected oat straws. In this connexion it is of interest to note that with each of samples 98 and 122 one of the seedlings not counted as *Ophiobolus* infected bore abundant runner hyphae without accompanying lesions on stem, leaf sheaths or roots. Presumably the straws tested in these instances bore the normal wheat strain, *O. graminis* proper, so that at least in sample 122 the two forms of the take-all fungus appear to have been present in the same wheat crop. It is concluded, therefore, that at least some of the take-all in all the wheat crops represented by the samples cited above was due to the so-called oat strain, *O. graminis* var. *Avenae*. This is borne out by the ascospore measurements for six of the samples, each of which indicates a mean length of 100 spores considerably above that of *O. graminis* proper.

Confirmatory results were obtained by Garrett with two additional samples of wheat straw from Dryfesdale and Morton parishes, Dumfriesshire, respectively. The former, from a wheat crop following oats and containing numerous take-all infected volunteer oat plants, gave positive reactions on oat

seedlings by the method cited above. The latter, from a first crop after old grass, bore perithecia with ascospores 85–121 μ long, mean length $106 \pm 1{\cdot}3\,\mu$, typical of *O. graminis* var. *Avenae*. Take-all was present to about 2 % in this wheat crop and to about the same extent in an adjacent oat crop with a similar history.

The writer is indebted to Dr C. E. Foister for facilitating the 1943 survey and for collecting several wheat samples in the east of Scotland, including number 98; to Messrs Rowatt, Young, Wilson, Laird and Grant, county organizers of Dumfriesshire, Kirkcudbrightshire, Wigtownshire, Ayrshire and Morayshire respectively, for placing their intimate local knowledge ungrudgingly at his disposal; and to Mr S. D. Garrett for encouragement and advice throughout.

Table 1. *Evidence of the existence of* Ophiobolus graminis *var.* Avenae *on wheat crops in Scotland*

Sample no.	Origin	Previous crop	Take-all in field	Ascospore mean μ	Size range μ	No. of seedlings infected by *Ophiobolus*	Total seedlings tested
25	Ringford, Kirkcudbrightshire	Oats ex lea	Under 1 %	104	85–115	17	20
27	Wigtown, Wigtownshire	Wheat	Trace	—	—	1	7
29	Wigtown, Wigtownshire	Oats	Under 1 : 1000	94·3	82–110	8	8
80	Ayr, Ayrshire	3 years' oats	Over 1 %	108	87–136	13	14
83	Old Luce, Wigtownshire	Spring wheat	Trace	104·4	93–116	4	4
85	Stevenston, Ayrshire	Green crop	Trace	109·6	91–128	11	11
89	Stranraer, Wigtownshire	Oats ex lea	Over 1 %	93·9	65–106	22	45
94	Ayr, Ayrshire	3 years' wheat	Over 1 %	—	—	6	9
98	Burntisland, Fife	Hay ex oats	Severe in patches	—	—	5	9
109	Duffus, Morayshire	Wheat ex oats	8 % and over	—	—	7	14
110	Dryfesdale, Dumfriesshire	Old lea	Under 1 %	—	—	5	8
114	Dryfesdale, Dumfriesshire	Oats	Nearly 1 %	—	—	9	20
115	Dryfesdale, Dumfriesshire	Oats	About 1 : 1000	—	—	9	11
122	Gretna, Dumfriesshire	Wheat	About 1 : 1000	—	—	16	20
123	Gretna, Dumfriesshire	Oats	About 1 : 1000	—	—	9	19
Culture from sample 89 above		—	—	—	—	81	100
Control, non-inoculated oat seed		—	—	—	—	0	100
10	Infected oat straw, Parton, Kirkcudbrightshire	Old lea	Over 1 %	—	—	13	19

REFERENCES

Dennis, R. W. G. (1943). Seedborne diseases in Scottish seed oats. *The Scottish Farmer*, 24 July.

Garrett, S. D. (1938). Decomposition of the resting mycelium of *Ophiobolus graminis* in infected wheat stubble buried in the soil. *Ann. appl. Biol.* **25**, 742.

Garrett, S. D. & Dennis, R. W. G. (1943). Note on the occurrence of *Ophiobolus graminis* var. *Avenae* E. M. Turner in Scotland in 1942. *Trans. Brit. mycol. Soc.* **26**, 146.

Turner, E. M. (1940). *Ophiobolus graminis* Sacc. var. *Avenae* var.n., as the cause of take-all or whiteheads of oats in Wales. *Trans. Brit. mycol. Soc.* **24**, 269.

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Mould deterioration of feeding-stuffs in relation to humidity of storage

Part I. The growth of moulds at low humidities

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(With Plate 6 and 6 Text-figures)

The literature relating to the growth of moulds at different humidities is surveyed.

Observations were made on the development of mould growth on six widely different types of feeding-stuffs for storage periods extending over some 3½ years. The main factors controlling mould growth were found to be:

1. *The relative humidity* rather than the moisture content of the food.
2. *The length of the storage period.* Mould growth took place relatively quickly on all feeding-stuffs stored at 100–75% R.H. Below 75% R.H. mould growth, while not prevented, only developed after a very prolonged latent period. Mould development was observed on locust beans exposed to a humidity as low as 65% after a latent period of over 2 years.
3. *The balance and type of nutrients provided* by the various feeding-stuffs was shown to influence the latent period as well as the extent of mould deterioration. This was confirmed by experiments on artificially dried grass fractions and by experiments on starch, protein and fibre mixtures.
4. *The temperature of storage* was found to affect the rate of mould development; mould growth developed sooner on samples stored at 22°C. than at 15·5°C.
5. *The type of mould species present.* At high humidities all mould species grew rapidly: at low humidities, only a few types were capable of growth.

Introduction

In normal times the supply and demand for animal feeding-stuffs could be adjusted to entail the holding of minimum stocks for limited storage periods. Under war conditions, however, the feeding-stuffs shortage has necessitated the maintenance of larger stocks for very much longer periods. Further, for reasons of security these stocks have had to be dispersed to country stores, such as improvised warehouses, sheds and farm buildings; many of these premises were not designed for storing feeding-stuffs and were inferior to the storage accommodation available at ports and at the place of manufacture. Even in pre-war years the storage of feeding-stuffs presented many problems in tropical and subtropical countries where the climatic conditions accelerated deterioration.

Deterioration of feeding-stuffs is usually associated either with a high atmospheric humidity or with a high moisture content in the stored material. Such deterioration may take the form of 'heating', which can lead to solidification of loose materials, loss of weight resulting from an increase in respiratory processes, darkening of colour or, in extreme cases, charring and possible spontaneous combustion. The presence of excess moisture will also encourage insect infestation, and it will allow deterioration by micro-organisms, of which damage by moulds is the most important. The palatability of such foods will be decreased along with their marketable value. Damaged feeding-stuffs are characterized by their loss of freshness, the presence of a musty smell, chemical breakdown of the food constituents by enzymes in the material itself or from the damaging micro-organisms, and by the production of injurious by-products.

The present paper is primarily concerned with the range of humidities at which mould species are able to develop on various feeding-stuffs. Observations on the extent and character of mould growth on different materials are also included, particularly in regard to the variation in nutrients which these provide. Detailed experiments on the water uptake of a large variety of feeding-stuffs exposed to a range of controlled humidities are included in Part II of this series, while observations on the different mould species isolated from the samples will be described in Part III.

Literature

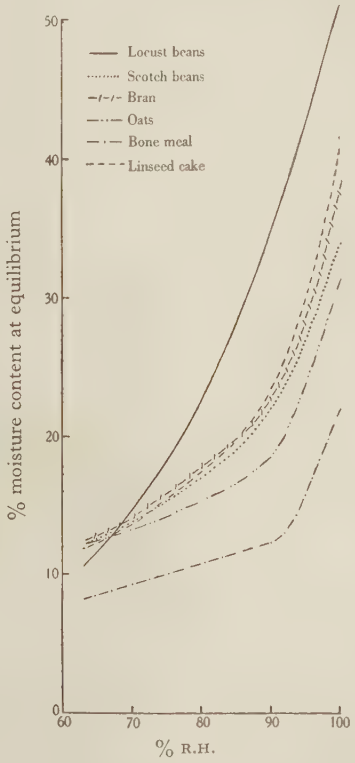
It has long been recognized that humidity and moisture content are the primary factors which control mould growth in stored products. McHargue (1920) found that moulds were able to develop on corn and corn meal of 15% moisture if no ventilation was provided, but at 12% moisture the material could be preserved undamaged for a long period. Thom & Le Fevre (1921) gave a critical figure of 13% moisture for mould growth on corn. Koehler (1938) recorded the growth of *Aspergillus glaucus* at a moisture content of 14·3%. He stated that other

species of moulds only developed at higher moisture contents and that, for commercial damage to corn, the moisture content must be 1½–2% above the minimum which permits mould growth. The growth of mould on cocoa beans of 8–9½% moisture content (equivalent to 82–87% R.H.) was observed by Bunting (1930).

The germination of mould spores on glass slides and nutrient agar exposed to controlled humidities was studied by Tomkins (1929), who found that, at any given temperature, the rate of germination was retarded as the humidity decreased. These observations, none of which was continued for longer than 20 days, were mostly carried out between 100 and 90% R.H., although a number of spores germinated at 83·8% R.H.—the lowest humidity recorded. Groom & Panisset (1933) found that the minimum humidity for the germination of conidia of *Penicillium chrysogenum* was 81% on glass and 72·6% on book-binding material. Galloway (1935) pointed out that the development of moulds on textiles was related to relative humidity rather than to moisture content. He suggested that the safe humidity for storage depended on the organisms liable to be present rather than on the nature of the material stored. Each mould species had a minimum moisture requirement, and members of the *Aspergillus glaucus* group, which were able to germinate at 75% R.H. within a 2-week period of storage, proved the most resistant to low humidities. The development of mould growth on samples of flour, bran and middlings exposed to a range of controlled humidities was investigated by Barton-Wright & Tomkins (1940), who stated that mould growth was prevented on flour exposed to 79% R.H. and on bran exposed to 75% R.H. Their experiments were, however, confined to a 4-month storage period. Macara (1943) found that moulds grew on dried meat exposed to 75% R.H., but that no growth took place on samples of meat exposed to 70% R.H. He suggested that the moisture content in equilibrium with 72·5% R.H. would give the maximum moisture content allowable if meat was to be free from moulding. Wright (1940) found that samples of artificially dried grass stored at 70% R.H. did not show signs of moulding for almost a year, after which period a sparse mycelium developed. He advised a safe limit of 67% R.H. for the storage of artificially dried grass, corresponding to a moisture content of roughly 13%. He states, however, that even this low value did not confer absolute immunity from mould growth when samples were stored for periods of 2 years or more.

Experimental method

Six feeding-stuffs were selected for a long period storage experiment in order to establish the relationship between humidity, moisture content and time of moulding. Linseed cake, bone meal, oats, scotch beans, bran and locust beans were chosen, as these feeding-stuffs showed a wide variety in water uptake at different fixed humidities. The moisture content-relative humidity equilibrium curves for these six feeding-stuffs are shown in Text-fig. 1. These six feeding-stuffs were, in addition, of widely different chemical composition and originated from a wide variety of plant or animal material. 0·4 g. (dry weight) samples of each of the feeds were spread in thin layers in small petri dishes. The dishes were placed on glass tripods and exposed in separate ground-glass stoppered museum jars, sealed with



Text-fig. 1. Moisture content-relative humidity equilibrium curves.

vaseline, to atmospheres of 100–60% R.H. The humidity was controlled by sulphuric acid solutions (Wilson, 1921): the specific gravity of the solutions was checked at the beginning and end of the experiments and no change was observed. All samples were stored at laboratory temperatures. The temperature variations (which were recorded by thermograph) never exceeded 10° C., a fluctuation which is too small to alter significantly the humidity inside the jars (Wilson, 1921). The mean variations were, of course, far smaller than this. The technique of exposing the feeding-stuffs in thin layers was chosen because it enabled rapid equilibrium to be obtained

between the moisture content of the feed and the humidity of the atmosphere, and facilitated accurate microscopic examination for mould development. Observations carried out with this technique closely simulate commercial storage conditions in so far as they represent conditions found on the surface layers of materials stored in sacks or on the ends and faces of oilseed cakes. It may be noted that in regard to moulding it is the condition of these surface layers which characterizes the appearance and quality of the stored material. The dishes were weighed daily until the moisture content-relative humidity equilibrium

ship between the time of appearance of mould mycelium and the relative humidity. Thus, after 10 days, mould mycelium appeared on different feeding-stuff samples whose moisture contents varied from 11 to 23% but whose humidities varied only from 80 to 82%, while, after 30 days, the equivalent figures for moisture content were 10–19% but for humidity only 76–78%. The variation in moisture content for any given feeding-stuff exposed to these two ranges of humidity amounts to only 2% for locust beans and to only 1% for any of the other five feeds.

TABLE 1. *Days before first appearance of mould mycelium on six selected feeding-stuffs*

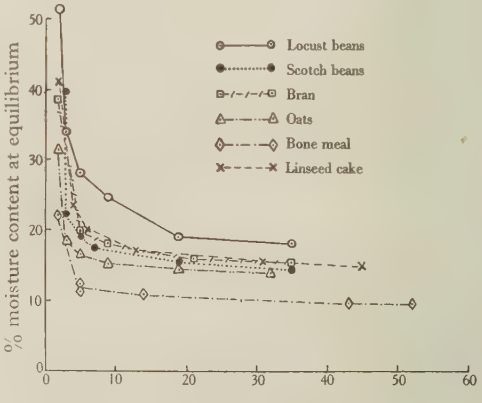
	% R.H.										
	100	90	85	80	77	75	72	71	67	65	63
Linseed cake	2	4	6	13	31	45	227	238	*	*	*
Bone meal	2	5	5	14	43	52	185	207	*	*	*
Oats	2	3	5	9	19	32	141	175	961	*	*
Scotch beans	3	3	5	7	19	35	118	129	525	*	*
Bran	2	4	5	9	21	35	101	129	525	*	*
Locust beans	2	3	5	9	19	35	82	118	314	861	*

* Mould mycelium had not developed on these samples after 1300 days of storage.

TABLE 2. *Days for development of mould fructification after appearance of mould mycelium on six selected feeding-stuffs*

% R.H.	Locust beans	Scotch beans	Bran	Linseed cake	Bone meal	Oats
100	2	1	1	2	2	1
90	3	2	2	2	4	2
85	4	2	4	5	12	8
80	7	5	9	8	7	12
77	22	20	20	30	39	50
75	29	30	38	66	65	49
72	59	57	65	299	676	720
71	32	46	46	48	654	686

was established and they were examined periodically under a low-power microscope for the appearance of mould mycelium and mould fructification. The number of days before development of these stages of deterioration was noted for each sample of feeding-stuff stored at the various humidities (see Tables 1 and 2).



Text-fig. 2. Rate of moulding in relation to the moisture content.

FACTORS INFLUENCING MOULD GROWTH

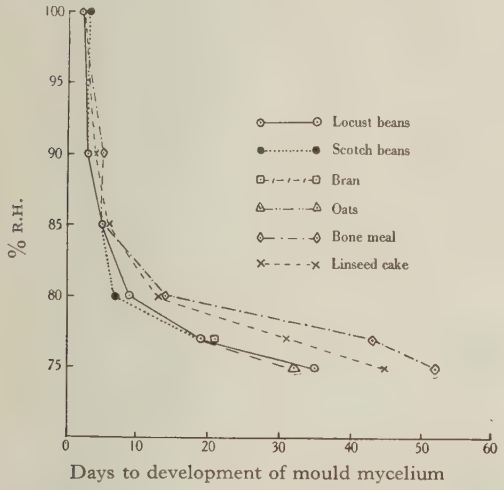
(1) *Humidity and moisture content*

The rate of mould development was found to depend on the relative humidity of the atmosphere rather than on the moisture content of the material stored. Although Text-fig. 2 indicates that for any one feeding-stuff there is a relationship between the time of the appearance of mould mycelium and the moisture content of the feed, such a relationship appears to exist only because the moisture content is itself a function of the relative humidity. Text-fig. 3 shows that there is a far closer and more consistent relation-

(2) *Duration of storage*

Moulds developed fairly rapidly on all samples stored at 100–75% R.H. (see Table 1). At humidities below 75% mould growth, while not entirely prevented, nevertheless only took place after a very prolonged latent period. Thus at 65% R.H. a latent period of over 2 years was required before spore germination took place on locust beans, as shown by the appearance of mould mycelium. This finding is in agreement with the results obtained on artificially dried grass by Wright (1940). It is, however, at

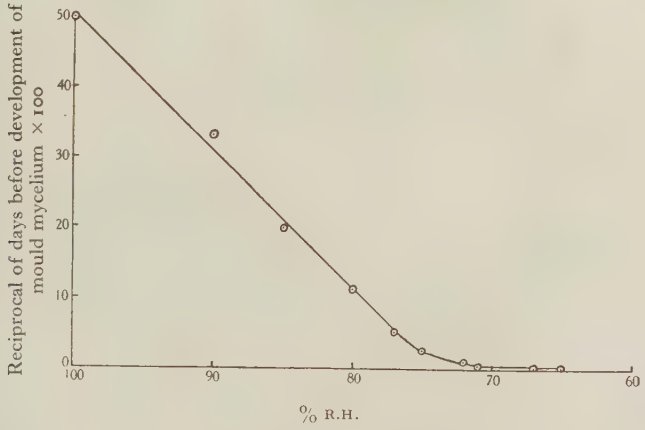
variance with the conclusions drawn by Barton-Wright & Tomkins (1940) who found that, when the reciprocals of the time taken to moulding were graphed, the relationship between the relative humidity and the rate of development of moulds on flour fell on a straight line. This line cut the zero axis at a relative humidity of 79%, and the authors assumed



Text-fig. 3. Rate of moulding in relation to humidity of storage.

that this figure could therefore be taken as the limiting humidity for mould growth. If the results obtained in the present work are similarly graphed (Text-fig. 4), the relationship between the relative humidity and the reciprocal of time to moulding only takes the form of a straight line between 75 and 100% R.H. Below 75% R.H. this straight line relationship no longer holds good: the curve tends sharply and assumes a more or less asymptotic form, growth taking place at as low a humidity as 65%. It might be argued that wheat flour, being a highly processed foodstuff consisting mainly of carbohydrate, does not provide as suitable a medium for the growth of moulds below 79% R.H. as do these six feeding-stuffs whose composition is more varied. In the present work mould development has, however, been observed in oats (67%* soluble carbohydrate) at humidities as low as 67% (see Table 1) and also in samples of pure rice starch† (less than 0·5% protein material) exposed to humidities of 75 and 72% (see Table 6). It seems probable that had Barton-Wright & Tomkins (1940) continued their experiments for longer than 123 days they, too, would have found that mould growth was not indefinitely prevented in flour exposed to humidities below 79%, or in bran exposed to humidities below 75%.

The most satisfactory method of expressing the relationship between the relative humidity and the time before the development of mould mycelium in long-period experiments is by means of logarithmic curves. Two such curves are shown in Text-fig. 5, i.e. those for locust beans and linseed cake which, of the six feeding-stuffs used, showed the most extreme differences in their liability to moulding (see Table 1). The appropriate points for samples of the other four feeding-stuffs stored at 72, 71 and



Text-fig. 4. 'Relative rate of development of moulds' on locust beans expressed by reciprocal of days before development of mould mycelium.

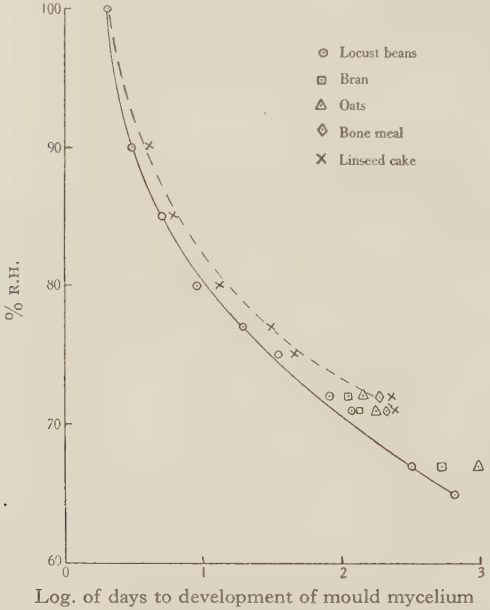
67% R.H. are included in this text-figure and fall within the two curves. Extrapolation from these curves would indicate that, if the same relationship between relative humidity and time taken for the

* Figure calculated as a percentage of the dry weight.

† The samples were inoculated with spores of *Aspergillus repens* (Corda) Saccardo.

development of mould mycelium continues to hold good at all humidities between 60 and 70%, the locust bean sample exposed to 63% R.H. would only produce mould mycelium after a latent period of 4 years and the linseed cake sample at 67% R.H. not before 13 years.

A second long-period storage experiment was set up in which the same general technique was employed, but in which the six feeding-stuffs were derived from different sources than those in the first experiment. This second series provided general confirmation of the findings of the first experiment, but differed in two respects. First, it was found in the second experiment that, at humidities of 80%



Text-fig. 5. Rate of moulding expressed by logarithmic curve.

and above, the length of time required before the first appearance of mould mycelium was roughly twice that found in the first experiment. This was probably due to the higher average temperature of the laboratory during the first week of the earlier experiment, which was carried out during the hot weather of June when the temperature registered between 70–80° F. During the corresponding week of the second experiment, which was conducted in January, the temperature of the laboratory was on the average some 10° F. lower. Secondly, at humidities of 72% and below, the time taken for the appearance of mould mycelium was, on the whole, shorter than in the first experiment. It seems likely that this difference was due to the fact that the feeding-stuffs used in the second series were obtained from older stocks than those used in the first experiment. Any mould spores present in the second batch of feeding-stuffs would thus have had a longer time to become acclimatized to conditions of low humidity before the start of the laboratory observations. In any storage experiments concerned with rate of mould growth it is important, therefore, to know the length and condition of previous storage of the feeding-stuffs used. Wherever possible fresh material should be employed.

(3) *Different mould species*

As shown in Table 1, differences were observed in the time taken for the appearance of mould mycelium on the samples of the six feeding-stuffs, even when these were exposed to the same humidities. Two explanations might account for such differences. First, the variety of mould species occurring in the different samples might have affected the rate of development of moulding. Reference has been made earlier in this paper to the suggestion of Galloway (1935) that mould development in textiles depends on the organisms liable to be present rather than on the nature of the material stored. Secondly, the type of feeding-stuff, and particularly the availability of the soluble nutrients, might have affected the time of germination of mould spores and the production of mould mycelium. The first explanation was studied by the use of aseptic methods. A long-period storage experiment was set up on the same lines as before except that the six feeding-stuffs and all the apparatus were sterilized by heat treatment. The feeding-stuffs were then inoculated with a spore suspension of *Aspergillus repens* (Corda) Saccardo. Observations had established that this species, one of the *Aspergillus glaucus* group, was the most ubiquitous of the mould species developing on feeding-stuffs and was able to germinate on some materials at humidities at least as low as 67%.

Between 100 and 80% R.H. no significant difference was shown in the rate of appearance of mould mycelium on the different feeding-stuffs between the sterilized-inoculated series and the normal unsterilized series. Between 80 and 70% R.H., however, the latent period showed less variability in the sterilized-inoculated series than in the normal series, but only to a small extent. In this connexion it must, however, be noted that at humidities of 100–80% mould development is so rapid that any significant difference in the time of appearance of mould mycelium in the two series of experiments would hardly have been measurable. Moreover, at humidities below 75%, the number of mould species which are able to develop is in any event very restricted even in an unsterilized feeding-stuff. It would appear, therefore, that the observed variations in the rate of mould development of the different feeding-stuffs were unlikely to have been due to the initial presence of different varieties of mould species.

(4) *Food nutrients*

It would appear more likely that the observed differences in the rate of moulding of the six feeding-stuffs were associated with the nutrients available for the germinating spores and young mycelium. Some feeding-stuffs, e.g. locust beans, provide a readily available source of soluble nutrients, while others, e.g. bone meal, are poor sources of food material for microbial activity. Of the six feeding-stuffs studied locust beans moulded quicker than any of the other substances below 80% R.H. and was the only medium on which mould growth took place at a relative humidity as low as 65% within the duration of the experiment, i.e. 1200–1400 days (see Table 1 and Pl. 6, fig. 1). Bran and scotch beans also moulded fairly rapidly and must be considered as materials capable of supporting mould growth below 75% R.H. Linseed cake, bone meal and oats, on the other hand, did not mould so quickly as the other materials and were only able to support mould growth to a limited extent at the lower humidities (75–65%) within the experimental period. Thus samples of linseed cake and bone meal exposed to 67% R.H. remained free from mould for over $3\frac{1}{2}$ years, while a latent period of $2\frac{1}{2}$ years was required before a fine mycelium developed on oats stored in this humidity.

It was also noticed that the extent of mould development varied according to the type of feeding-stuff. Samples providing a supply of readily available nutrients, e.g. locust beans, developed a denser mould mycelium and fructification than samples which were not so nutritious, e.g. bone meal. Three criteria have been put forward (Tomkins, 1932), for the measurement of the rate of germination of mould spores: (1) the latent period of germination, (2) the percentage germination, (3) the rate of elongation of the germ tubes. The application of these criteria to observations on the moulding of textiles was considered by Galloway (1935), who stated that the percentage germination of mould spores on textiles was difficult to measure and that, as the third criterion varied with the first when only the moisture requirements of moulds were under test, the most satisfactory measurement was the latent period of germination. At the start of the present work on mould deterioration of feeding-stuffs it was realized that, as it was not possible to study the behaviour of the individual mould spores present on the feeds, neither the percentage germination nor the rate of elongation of the germ tubes could be measured. It was possible, however, to measure the latent period before the appearance of mould mycelium and also before the appearance of mould fructification. While figures obtained in this way gave some indication of the susceptibility of different materials to mould growth at various humidities, they gave little information as to the rate of spread of the mould which is of importance in relation to the extent of deterioration caused. Thus, the same value might sometimes be recorded for a few traces of developing mould mycelium as for general mycelial growth over the whole surface of the material. Similarly, the same value might be stated for the appearance of an isolated spore head as for general development of mould fructification throughout the material. This was of particular significance at humidities of 80% and above. An indication of the relative rate of mould growth on the different feeding-stuffs was, however, obtained by comparing the number of days required for the development of mould fructification after the initial appearance of mould mycelium. It was then found that the feeding-stuffs which provided a readily available supply of soluble nutrients, e.g. locust beans, caused a rapid development of a dense mould mycelium soon after spore germination, and that fructifications were quickly produced. Typical mould fructifications are shown in Pl. 6, fig. 4. If the feeding-stuff provided a less abundant source of such nutrients, e.g. oats, the growth of mould mycelium was slower and the time before the appearance of fructifications longer. The values obtained in this way, which are shown in Table 2, confirm the earlier conclusion that locust beans, bran and scotch beans can more readily support mould growth than linseed cake, bone meal and oats. This fact is of considerable importance in regard to the commercial storage of feeding-stuffs, since it is known that, while sparse mould development has little deteriorating effect on feeds, vigorous mould growth and abundant production of spores not only causes a breakdown and loss of valuable feeding material (sometimes accompanied by the production of injurious by-products), but also results in the rapid dissemination of the moulds to other materials.

The rate of mould development on artificially dried grass fractions

In order to examine this aspect of the subject in greater detail it was decided to study the development of moulds on a sample of artificially dried grass sieved into three separate fractions according to their degree of fineness. This material had the advantage, for the purpose of comparison, of being derived from the same source: the three fractions had similar initial moisture contents, the moisture content-relative humidity equilibrium curves were of almost identical type, and the mould species initially present were the same. On the other hand, the amount of readily available nutrients as measured by carbohydrate, fat and protein varied for each of the three fractions (Table 3), while the availability of these nutrients to the mould spores may also have varied with the coarseness of the fraction and the nature of the fibrous coating of the particles. Thin layers of the materials were exposed to humidities of 100–60% as in previous experiments and the number of days

before the development of mould mycelium (Table 4) and mould fructification were noted. It was found that below 80% R.H. the development of mould mycelium was slowest with the coarse sample (Table 4), while at humidities of 80% and above mould fructification occurred most rapidly with the fine sample (Table 5).

TABLE 3. *Analysis of three dried grass fractions*

Fraction	Moisture %	Soluble carbohydrate %	Fat %	Protein %	Fibre %	Ash %
Coarse	9·2	46·2	2·9	14·5	19·8	7·5
Intermediate	9·1	45·1	3·4	17·7	16·6	8·1
Fine	8·9	44·8	4·7	18·8	13·9	8·9

Tomkins (1929) showed that the spores of some mould species were able to germinate on plain glass surfaces exposed to certain humidities, while other species required a supply of nutrients to effect germination. He also pointed out that some species germinated at a greater rate when nutrients were supplied, and that the range of humidity over which germination was possible was also extended; germination occurred at lower humidities when nutrients were available. Observations carried out in the present series of experiments on the growth of moulds on the three dried grass fractions, on the starch, protein and fibre mixtures and also on the six feeding-stuffs, fully confirm these conclusions. The shorter period before the development of mould mycelium, as well as the quicker rate of mould fructification on the finer dried grass fractions indicate that the latter furnished a better supply of nutrients for spore germination. The fineness of the state of division and the nature of the fibrous coating of the particles may also have affected the availability of these nutrients to the developing mould species. It is also clear that spore germination and mycelial

TABLE 6. *Days before first appearance of mould mycelium on starch, protein and fibre mixtures*

	% R.H. 100	90	85	80	75	72	70
Starch + protein	3	4	15	31	66	86	151
Starch	3	9	25	46	65	100	*
Protein	6	12	24	28	56	126	*
Starch + fibre	3	7	19	54	120	129	*
Fibre	8	11	43	131	*	*	*

* Mould mycelium had not developed on these samples after 226 days of storage.

TABLE 4. *Days before first appearance of mould mycelium on three dried grass fractions*

Fraction	% R.H. 100	90	85	80	77	74	72	70	67	64	60
Coarse	2	4	8	12	55	68	155	166	867	*	*
Intermediate	4	4	8	15	55	61	135	148	867	*	*
Fine	4	4	8	17	41	44	83	131	761	867	*

* Mould mycelium had not developed on these samples after 1200 days of storage.

TABLE 5. *Days for development of mould fructification after initial appearance of mould mycelium on three dried grass fractions*

% R.H.	Fractions: Fine	Intermediate	Coarse
100	1	2	5
90	3	5	7
85	4	7	9
80	6	14	25

TABLE 7. *Days for development of mould fructification after appearance of mould mycelium on starch, protein and fibre mixtures*

% R.H.	Protein	Protein + starch	Starch	Starch + fibre	Fibre
100	1	1	1	1	2
90	1	3	5	4	5
85	1	3	6	10	13
80	1	8	12	17	38
75	2	28	13	17	*
70	0	54	126	98	*

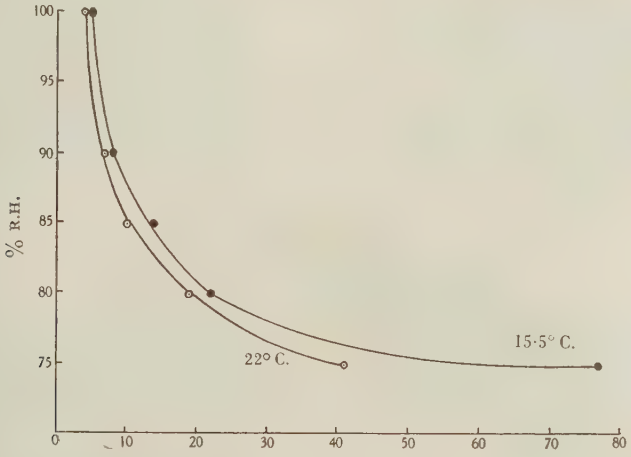
* Neither mould mycelium nor fructification had developed on these samples after 226 days of storage.

The rate of mould development on starch, protein and fibre mixtures

The effect of food nutrients on mould development was also studied by exposing samples of rice starch, egg albumen and the fibre constituent of bran* to a range of controlled humidities at 25°C. The samples were inoculated with spores of *Aspergillus repens* (Corda) Saccardo. The rate of mould development on these three materials and on mixtures of them was observed. Mould mycelium developed most rapidly on samples where a balance of nutrients was provided, i.e. on the starch-protein mixtures, while samples containing the fibre constituent, took much longer periods for mould mycelium to develop (Table 6). The rate of development of mould fructification appeared to depend, on the other hand, on the amount of protein present, the samples of pure protein giving the most rapid development (Table 7).

* Extracted according to the A.O.A.C. (1941) method for crude-fibre analysis.

development was possible at lower humidities where there was a greater supply of available nutrients. Thus, only the finest fraction of dried grass was able to support mould growth at 64% R.H. within the duration of the experiment (1200 days). Of the starch, protein and fibre mixtures the samples which provided a balance of food nutrients, i.e. the starch +protein mixtures, developed mould mycelium most rapidly and, within the period of the experiment, were able to support mould species at lower humidities than any of the other mixtures. Similar conclusions can also be derived from observations on the six different feeding-stuffs, of which locust beans supported the development of mould mycelium at lower humidities than bran, scotch beans or oats, which in turn enabled mould growth to take place at lower humidities than bone meal or linseed cake.



Text-fig. 6. Effect of temperature on mould growth.

For these feeding-stuffs it would appear that a readily available source of soluble carbohydrate is particularly necessary for the support of mould growth at low humidities. Thus locust beans, with 81%* soluble carbohydrate and only 6% protein, were able to support mould growth at 64% R.H. Oats, bran and scotch beans with 67, 64 and 57% soluble carbohydrate and 12, 16 and 30% protein, respectively, were able to support mould growth at 67% R.H. but not at 64% R.H. Linseed cake and bone meal, whose analysis showed only 40 and 5% soluble carbohydrate and as much as 35 and 32% protein, respectively, remained free from moulding at humidities of 67% and below.

(5) *Temperature*

Barton-Wright & Tomkins (1940) found that at constant humidity the rate of appearance of mould growth increased as the temperature was decreased. It was stated earlier in this paper that an increase in the average temperature recorded in the laboratory accelerated the rate of mould development on samples stored at humidities of 80% and above. The effect of temperature on the development of mould growth under more carefully controlled conditions was briefly studied by exposing samples of locust beans and oats to humidities of 100–75% at 15·5 and 22° C. for 100 days.

The moisture content-humidity equilibrium curves for each feeding-stuff stored at these two temperatures showed a small difference owing to the fact that the amount of water held at 15·5° C. was slightly greater than at 22° C. This agrees with Gane's (1941) finding. The samples stored at 22° C. developed mould mycelium sooner than those stored at 15·5° C., and mould growth proceeded more rapidly at the higher temperature (see Table 8 and Text-fig. 6).

Table 8. *Effect of temperature on mould growth—locust beans**

	Days before appearance of mould mycelium		Days for development of mould fructification after initial appearance of mould mycelium	
% R.H.	15·5° C.	22° C.	15·5° C.	22° C.
100	5	4	7	5
90	8	7	12	8
85	14	10	22	15
80	22	19	37	24
75	77	41	More than 100 days	64

* A similar relationship was found for oats.

* Figures calculated as a percentage of the dry weight.

It is common experience in commercial storage to find that feeding-stuffs are more susceptible to mould deterioration during the summer months when the average atmospheric temperature is higher than during the winter months. The importance of exercising special care in maintaining summer storage conditions at a level of humidity which will not encourage mould growth is clear. Such precautions are, of course, also specially necessary in the storage of foodstuffs in tropical and subtropical climates.

The authors wish to express their gratitude to Mr George Smith of the London School of Hygiene and Tropical Medicine for his help in identifying the mould used in the sterilized-inoculated series.

REFERENCES

Barton-Wright, E. C. & Tomkins, R. G. (1940). The moisture content and growth of mould in flour, bran and middlings. *Cereal Chem.* **17**, 332.

Bunting, R. H. (1930). Research on infestation of stored products. II. Mycological aspects. *Ann. appl. Biol.* **17**, 402.

Galloway, L. D. (1935). The moisture requirements of mould fungi with special reference to mildew in textiles. *J. Text. Inst., Manchr*, **26**, T 123.

Gane, R. (1941). The water content of wheats as a function of temperature and humidity. *J. Soc. chem. Ind., Lond.*, **60**, 44.

Groom, P. & Panisset, T. (1933). Studies on *Penicillium chrysogenum* Thom, in relation to temperature and relative humidity of the air. *Ann. appl. Biol.* **20**, 633.

Koehler, B. (1938). Fungus growth in shelled corn as affected by moisture. *J. agric. Res.* **56**, 291.

Macara, T. J. R. (1943). The growth of moulds on dried meat. *J. Soc. chem. Ind., Lond.*, **62**, 104.

McHargue, J. S. (1920). The cause of deterioration and spoiling of corn and corn meal. *J. industr. Engng Chem.* **12**, 257.

Thom, C. & Le Fevre, E. (1921). Flora of corn meal. *J. agric. Res.* **22**, 179.

Tomkins, R. G. (1929). Studies on the growth of moulds. *Proc. roy. Soc.* B **105**, 375.

—— (1932). Measuring germination. *Trans. Brit. mycol. Soc.* **17**, 147.

Wilson, R. E. (1921). Humidity control by means of sulfuric acid solution with critical compilation of vapor pressure data. *J. industr. Engng Chem.* **13**, 326.

Wright, N. C. (1940). The storage of artificially dried grass. *J. agric. Sci.* **31**, 194.

EXPLANATION OF PLATE 6

Photomicrographs illustrating extent of mould development on locust beans after $3\frac{1}{2}$ years' storage at various humidities. (× 50.)

Fig. 1. Storage at 65 % r.h. Sparse mycelium.

Fig. 2. Storage at 67 % r.h. Generalized network of mycelium.

Fig. 3. Storage at 71 % r.h. Dense mycelium with immature perithecia of *Aspergillus ruber* (Spieckermann & Bremer).

Fig. 4. Storage at 72 % r.h. Dense mycelium and conidial fructifications of *Aspergillus repens* (Corda) Saccardo.

(*Received* 30 *November* 1943)

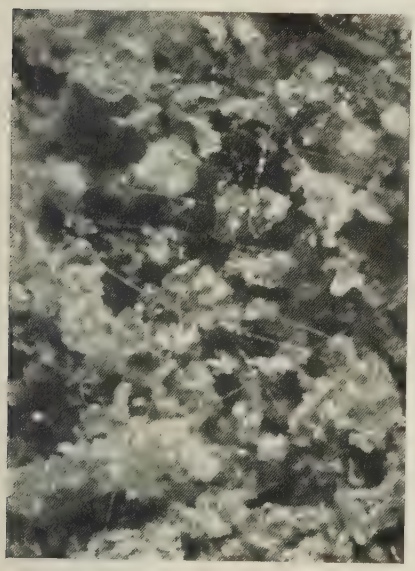


Fig. 1

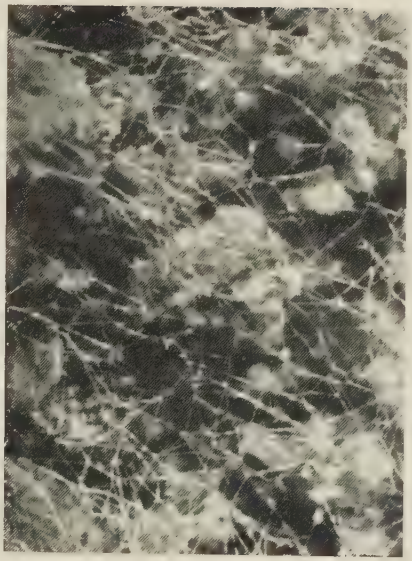


Fig. 2

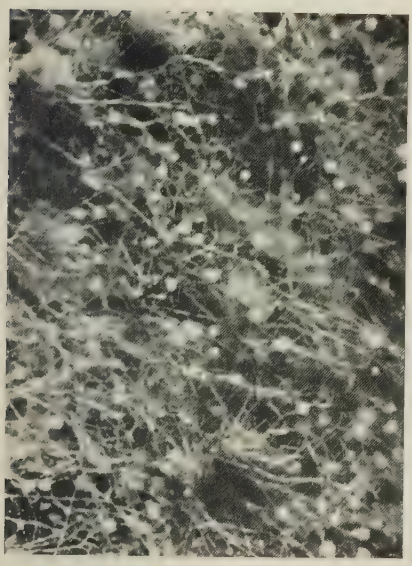


Fig. 3

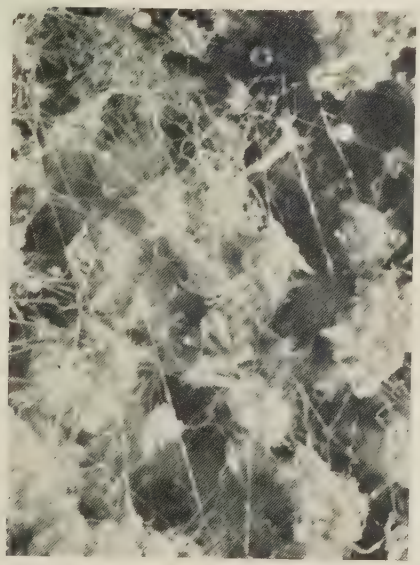


Fig. 4

SNOW, CRICHTON AND WRIGHT—MOULD DETERIORATION OF FEEDING-STUFFS IN RELATION TO HUMIDITY OF STORAGE

Mould deterioration of feeding-stuffs in relation to humidity of storage

Part II. The water uptake of feeding-stuffs at different humidities

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(With 5 Text-figures)

The water uptake of a wide variety of feeding-stuffs was determined over a range of fixed humidities. It was shown that the level and shape of the water uptake curves are closely related to the amounts of soluble carbohydrate and protein present. Fibre exerts a depressing effect on the water uptake, while the value is also lowered by the presence of inert fats and non-hygroscopic ash constituents. These conclusions were confirmed by direct experiments on the water uptake of samples of pure starch, protein and fibre.

The results of the investigations have enabled safe limits of moisture content to be laid down, for both short- and long-period storage, below which mould growth will not normally take place. These limits vary for each individual feed, representative figures being included in the paper in tabular form.

Introduction

In Part I of this series (Snow *et al.* 1944) it was shown that the occurrence of mould growth in six typical feeding-stuffs was primarily a function of the relative humidity. Accurate measurements of the relative humidity of commercial stores are, however, seldom made, and the daily variations in humidity are, in any event, often so great as to render any such measurement of very limited value. Moreover, when feeding-stuffs are stored in sacks or in bulk, the relative humidity of the air in the interstices between the individual food particles is probably as important as that of the surrounding atmosphere, since mould growth is not confined to the surface layers of material but is liable to take place throughout the mass.

In commercial practice it is, however, relatively easy to obtain an accurate measure of the moisture content of bulked feeding-stuffs. Since for any individual feeding-stuff the water uptake is directly related to the relative humidity, and since the maximum humidities at which different feeds will remain free from moulding can now be derived from the data in Part I, a knowledge of the relationship between relative humidity and water uptake should enable the conditions for safe storage to be assessed directly in terms of moisture content. The present paper gives details of the water uptake of a wide variety of feeding-stuffs corresponding to a range of fixed humidities. From such data the maximum moisture content for the safe storage of each individual feeding-stuff has been calculated. An incidental study has been made of the relation of water uptake to the proximate constituents of the feeding-stuffs used in the investigation.

Literature

Bailey (1920) obtained simple parabolic curves for the degree of water uptake of 'patent' and 'clear' flours. Wilson & Fuwa (1922) studied the humidity equilibria of a variety of common substances including textiles and certain foodstuffs. Coleman & Fellows (1925) found that for cereals and flax seed hygroscopic moisture did not increase uniformly with increasing atmospheric humidity. The water uptake could be represented by a graph which was characteristic for each material and which showed a greater change between 75 and 90% R.H. than between 40 and 70% R.H. Fairbrother (1929) exposed samples of flour to a range of controlled humidities and found that from 15 to 90% R.H. a straight-line relationship existed between the relative humidity and the water uptake. This relationship was independent of the original moisture content of the material. Beyond these humidity limits the character of the relationship changed. Rozsa (1935), reviewing the work of L. Pap, gave figures for the water uptake of wheat exposed to different humidities. Gane (1941) studied the water relations of wheat at different temperature levels and found that equilibrium was reached quicker at the higher temperatures. The water content of wheat in equilibrium with a fixed humidity was highest at the lowest temperature.

Experimental

The technique employed in measuring the water uptake of thin layers of feeding-stuffs exposed to a range of fixed humidities was described in Part I. The samples of each feeding-stuff used in the present

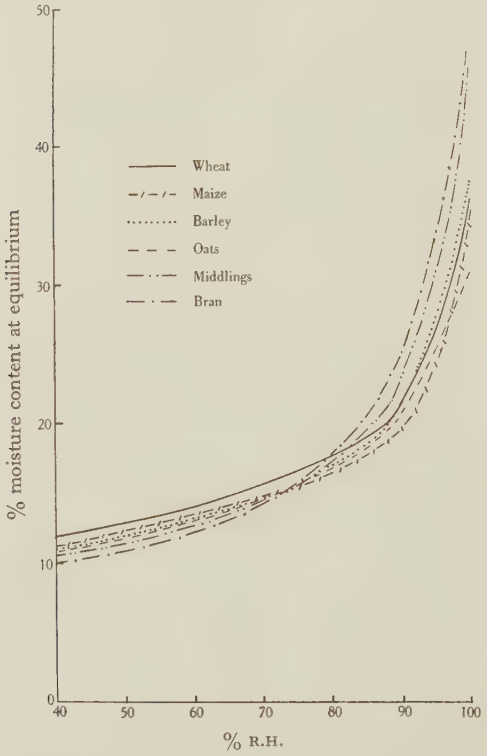
series of experiments were exposed to a range of humidities between 40 and 100% and were weighed daily until equilibrium had been established. Equilibrium was attained with all samples except those exposed to 90 and 100% R.H., where the development of moulding intervened.

Results

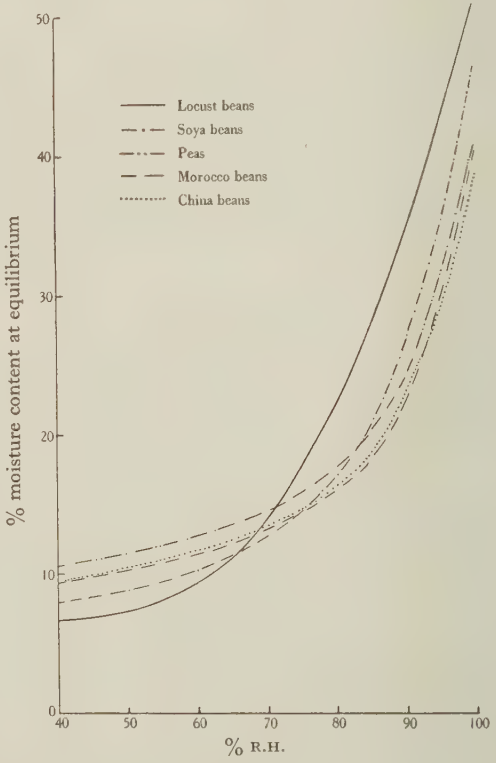
(*a*) *Water uptake of individual feeding-stuffs*

It has been observed by several investigators that water uptake curves tend to exhibit hysteresis, i.e. the curves for hydration and dehydration are not identical. Since in the present series of experiments the main object was to simulate the conditions typical of commercial practice, no attempt was made to differentiate between hydration and dehydration equilibria: the samples of feeding-stuffs as received were directly exposed to the various humidities without any preliminary adjustment of moisture content. The present water uptake curves will therefore represent hydration values for atmospheres of higher humidity than that corresponding to the initial moisture content of the feeding-stuff, and dehydration values for atmospheres of lower humidity. The moisture contents at each humidity have been expressed as percentages of the equilibrium weights.

The relationships between the relative humidities and the water uptakes of a wide variety of feeding-stuffs are shown in Text-figs. 1–4. It will be seen that these relationships take the form of a series of smooth curves, each curve being characteristic of the individual feed. Small variations were found between different samples of the same feeding-stuff. There are four possible explanations for this: (1) the differences may be due to slight variations in the composition of different samples of the same feeding-stuff; (2) variations may have occurred as a result of hysteresis, particularly where samples of very different initial moisture content were used; (3) slight variations in the processing treatments to which different batches are subjected may have affected the capacity for water uptake; (4) it was found that the temperature of storage affected the equilibrium value; the samples stored at a lower temperature were able to retain a slightly greater amount of water in equilibrium with a fixed humidity than similar samples kept at a higher temperature (see also Gane,

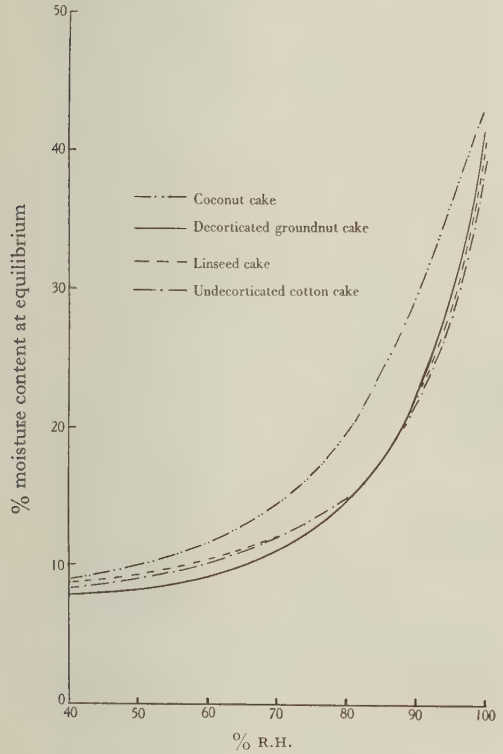


Text-fig. 1. Moisture content-relative humidity equilibrium curves. Cereals and cereal by-products.

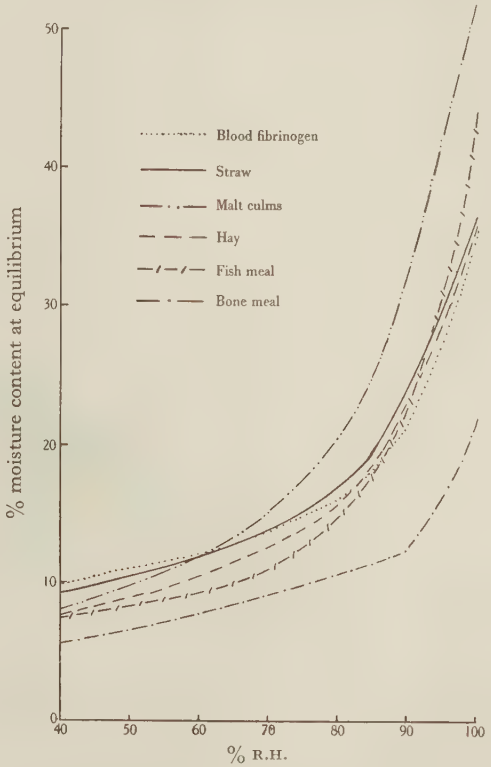


Text-fig. 2. Moisture content-relative humidity curves. Legumes.

1941). However, the summation of these variations seldom amounted to more than 0·5% moisture at any given humidity. The curves vary, however, not only in general level but also in shape. Thus between 40 and 80% R.H. the curves for cereals (Text-fig. 1) show higher values than those for legumes (Text-fig. 2), which in turn exceed those for oil cakes (Text-fig. 3). On the other hand, at humidities above this range the position is reversed, oil cakes and legumes showing higher water uptakes than cereals.



Text-fig. 3. Moisture content-relative humidity equilibrium curves. Oil cakes.



Text-fig. 4. Moisture content-relative humidity equilibrium curves. Miscellaneous feeding-stuffs.

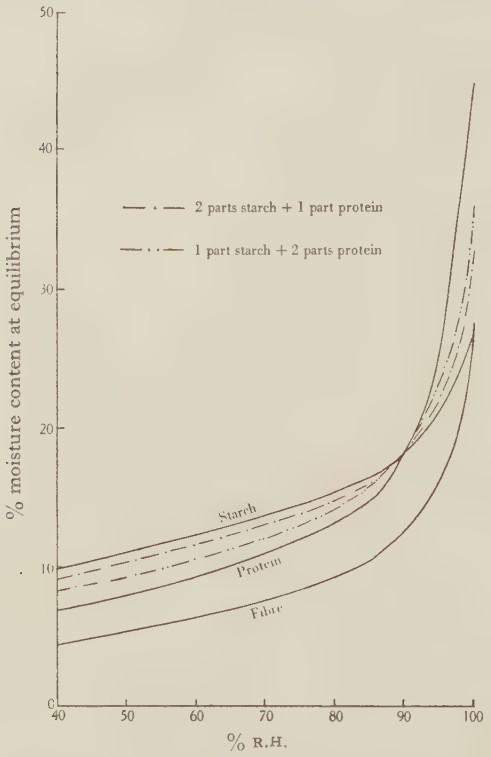
Differences in both the level and shape of the curves are found not only between the different classes of feeding-stuffs but between individual feeds within a single class. Thus, at humidity levels below 80% whole cereals give higher water uptakes than cereal by-products (bran and middlings), but above 80% the curves cross, the by-products giving the higher figures. Similar differences are shown among the legumes and the oil cakes, the most marked instances of crossing-over being observed with soya beans for the legumes and decorticated ground-nut cake for the oil cakes. Coconut cake and locust beans give exceptional curves, coconut cake showing a remarkably high water uptake over the full range of humidities studied, and locust beans giving a curve of quite abnormal steepness. Text-fig. 4 shows the widely varying water uptakes of a variety of feeds of miscellaneous origin.

It seemed clear that the above differences in the level and shape of the water uptake curves must be attributable to differences in the proximate composition of the feeds. From the point of view of water uptake fat may be considered inert, while the constituents of the ash (unless these are of an exceptionally hygroscopic nature) are also unlikely to affect the water uptake materially). The relatively inert influence of a high ash content is well illustrated by the results obtained with bone meal (Text-fig. 4) which gave by far the lowest level of water uptake of all the feeds studied, and which contained some 55% of ash constituents. There remain the soluble carbohydrate, protein and fibre fractions. Two methods have been used to ascertain the influence of these fractions: (*a*) direct determinations have been made of the water uptake of purified starch, protein and fibre and of typical mixtures of such

materials, and (*b*) the general relationship between the proximate composition and the water uptake of the various feeds has been studied.

(*b*) *Water uptake of purified materials*

Rice starch, two plant proteins (edestin and glutenin), and purified fibre obtained from bran* were used for the water uptake determinations. The same general technique was adopted as with the feeding-stuffs, except that the materials were pre-dried by exposure in a desiccator at 0% relative humidity. Both hydration and dehydration curves were obtained over the full range of 0–100% relative humidity. The differences between the hydration and dehydration curves (though fairly marked with glutenin, as might be expected from its known ability to retain moisture and its consequent importance in bread-making) were insufficient to affect the general nature of the results.



Text-fig. 5. Moisture content-relative humidity equilibrium curves. Starch protein and fibre mixtures

* Extracted according to the A.O.A.C. (1941) method for crude fibre analysis.

Typical hydration curves for the purified materials (rice starch, glutenin* and fibre) over the range 40–100% R.H. are shown as continuous lines in Text-fig. 5. It will be seen that the curves are of two general shapes. The starch (representing soluble carbohydrate) gives a straight-line relationship from below 40% to 85% R.H. Over this range the starch curve lies well above the protein curve. Above 85% R.H. the slope of the starch curve sharpens, but the water uptake at 90–100% humidity only reaches about 27%. The protein curve, on the other hand, gives a relatively steep slope at humidities above 75%, and while it starts well below the starch curve at 40%, it crosses it at about 90% humidity and ultimately gives the exceptionally high water uptake of 40–50% at humidities between 90 and 100%. It may be noted that this type of steep curve appears to be a general characteristic of proteins, since experiments carried out with the animal proteins, casein and egg albumen, gave similar results, though with a far more marked rise in water uptake at high humidities. The curve of the purified fibre is similar in shape to that of the starch, but the level of water uptake is exceptionally low throughout practically the whole range of humidities.

It would appear from these results that the shape of the water uptake curve of a feeding-stuff will depend primarily on the relative proportions of soluble carbohydrate and protein which it contains. The level will depend on the absolute quantities of these two constituents present in the feed. The only effect likely to be caused by the presence of a high proportion of fibre will be to depress the general level, without materially altering the shape.

These facts are clearly illustrated in Text-fig. 5, where the curves of two composite mixtures have been graphed as broken lines. It will be seen that the slopes of these two curves are governed by the starch-protein ratio. Thus, at low humidities, both mixtures give curves below the pure starch but above the pure protein: at high humidities the position is reversed, the mixtures giving curves well above the pure starch but well below the pure protein. The four curves, pure protein, 2 parts protein + 1 part starch, 2 parts starch + 1 part protein, and pure starch show, in fact, the same crossing-over phenomenon which has already been found to be typical of the intact feeding-stuffs.

(*c*) *Relation of water uptake to proximate composition*

An examination of the proximate composition of the various classes of feeding-stuff confirms the conclusion that the relative proportions of soluble carbohydrate and protein play the largest part in determining the level and shape of the water uptake

* Edestin gave a hydration curve of similar shape to glutenin, though it was slightly more hygroscopic.

curve. Typical analytical figures, calculated for simplicity on a moisture, fat and ash-free basis, are shown in Table 1.*

If the mean water uptakes of the four classes of feed at 63 % R.H. are considered, it will be seen that these vary directly with the soluble carbohydrate content and inversely with the protein content. At this relative humidity the high proportion of starch raises, and the high proportion of protein depresses, the water uptake. At 90% R.H. the position is completely reversed: the water uptakes vary directly with the protein content and inversely with the content of soluble carbohydrate. At this relative humidity the high proportion of protein raises, and the high proportion of soluble carbohydrate depresses, the water uptake. It is true that the average figure for oil cakes at 90 % R.H. falls below that of both the cereal by-products and the legumes. This is, however, probably due to the effect of the relatively higher content of fibre and inert constituents (fat and ash) in the oil cakes (24 % of the total constituents) in comparison with the cereal by-products (17½ %) and the legumes (14½ %).

Table 1. *Relation between water uptake and proximate constituents* of four classes of feeding-stuffs*

	Mean water uptake at		Soluble carbohydrate	Protein	Fibre
	63 % R.H.	90 % R.H.	%	%	%
Cereals	13·9	19·9	80	14·5	6
Cereal by-products	13·0	23·9	70	21	9
Legumes	11·9	24·4	55	37	9
Oil cakes	10·5	22·0	42	43	14

* Figures calculated on a moisture, fat- and ash-free basis.

It may be noted that this general finding is equally applicable to the curves of individual feeds. Thus with legumes the soya beans, and with oil cakes the decorticated ground-nut cake, show the lowest water uptakes at low humidities and the highest water uptakes at high humidities. Both these feeds contain exceptionally high proportions of protein (55–57 %) and exceptionally low proportions of soluble carbohydrate (37–39 %). It must, of course, be recognized that the nature of the soluble carbohydrate constituents and the type of plant protein will play some part in determining the shape of the water uptake curves of individual feeding-stuffs. Moreover, the general level of the curves will, in addition, be affected by the proportions of inert material (fat and non-hygroscopic ash), as has already been noted in regard to the curve for bone meal. It is, however, clear from the above discussion that the relative proportions of soluble carbohydrate and protein play the most important part in determining the water uptake of the feeding-stuffs used in the present trials.

* Coconut cake and locust beans have been omitted in calculating the average values in Table 1, owing to the very exceptional nature of their water uptake curves.

Practical applications

It was pointed out in Part I of this series that the liability of feeding-stuffs to mould growth depends on several factors, of which the relative humidity and the length of the storage period are probably the most important. These moulding trials showed that for the short-period (3 months) storage of the most nutritious* of the feeds studied, i.e. locust beans, the relative humidity ought not to exceed 72 %, while for long-period storage (up to 2–3 years) a figure as low as 65 % R.H. must be accepted as the safe maximum. These limits refer to storage periods at normal temperatures (60–70° F.): at higher temperatures the period of safe storage would be correspondingly reduced.

Table 2. *Safe levels for storage of feeding-stuffs, below which mould growth will not normally take place*

	% moisture	
	For short-period storage	For long-period storage
Cereals and cereal by-products:		
Wheat	15·7	14·6
Maize	14·8	13·7
Barley	14·8	13·6
Oats	14·5	13·4
Middlings	14·4	13·1
Bran	14·4	12·8
Legumes:		
Locust beans	15·1	11·3
Peas	14·7	13·3
China beans	13·9	12·7
Scotch beans	13·9	12·4
Morocco beans	13·5	12·0
Soya beans	13·3	11·4
Oil cakes:		
Coconut cake	15·1	12·6
Linseed cake	12·3	11·0
Undecorticated cotton cake	12·3	11·0
Undecorticated ground-nut cake	12·3	11·0
Palm kernel cake	11·5	10·2
Decorticated ground-nut cake	11·5	9·8
Miscellaneous feeds:		
Malt culms	15·3	12·6
Straw	14·8	12·8
Blood fibrinogen	14·1	12·9
Artificially dried grass	13·7	11·1
Hay	12·6	11·0
Fish meal	11·5	9·9
Distillers' grains	11·0	9·8
Meat and bone meal	10·3	8·7
Bone meal	9·5	8·4

* The term 'nutritious' here implies the value of the nutrients as a basis for mould growth, and not the general feeding value for livestock.

From the curves given in Text-figs. 1–4 it is possible to obtain the moisture contents of any of the more common feeding-stuffs which are in equilibrium with either of these two humidity levels. For convenience the relevant figures are set out in Table 2. It will be seen that the safe moisture contents for short-period storage range from 9·5% for bone meal to 15·7% for wheat, while the corresponding figures for long-period storage are about 1% lower, i.e. 8·4 and 14·6% respectively. The values for other feeding-stuffs fall intermediately.

It must be emphasized that the figures in Table 2 only refer to the moisture limits below which mould growth will not normally take place. Moulding is, however, only one form of storage deterioration. It is well recognized that feeds containing a high percentage of moisture are specially liable to insect infestation, as well as to spoilage by 'heating'. These facts must be taken into account in applying the figures in Table 2 to commercial storage conditions.

The authors are indebted to Dr E. C. Owen for the analytical figures used in the present study.

REFERENCES

Bailey, C. H. (1920). The hygroscopic moisture of flour exposed to atmospheres of different relative humidity. *J. industr. Engng Chem.* **12**, 1102.

Coleman, D. A. & Fellows, H. C. (1925). Hygroscopic moisture of cereal grains and flaxseed exposed to atmospheres of different relative humidity. *Cereal Chem.* **2**, 275.

Fairbrother, T. H. (1929). The influence of environment on the moisture content of flour and wheat. *Cereal Chem.* **6**, 379.

Gane, R. (1941). The water content of wheats as a function of temperature and humidity. *J. Soc. chem. Ind., Lond.*, **60**, 44.

Rozsa, T. A. (1935). Drinking habits of the wheat berry. *Northw. Miller*, **184**, 73.

Snow, D., Crichton, M. H. G. & Wright, N. C. (1944). Mould deterioration of feeding-stuffs in relation to humidity of storage. Part I. The growth of moulds at low humidities. *Ann. appl. Biol.* **31**, 102.

Wilson, R. E. & Fuwa, T. (1922). Humidity equilibria of various common substances. *J. industr. Engng Chem.* **14**, 913.

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Serological studies on sugar-beet yellows virus

By A. Kleczkowski and M. A. Watson

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Specific antisera have been prepared against the sap expressed from beet plants infected with beet yellows virus.

The antigen is unstable. In the sap it is destroyed by keeping for 2–3 days at room temperature or by heating for 10 min. at 52°C. It is unaffected by *p*H changes between 5 and 9. In detached leaves at room temperature it remains unchanged for at least 6 days, whereas the ability of aphides to transmit from these leaves fell considerably in 4 days.

The antigen can be reversibly precipitated by ammonium sulphate or sedimented by high-speed centrifugation. However, all the attempts to isolate it from other sap constituents or concentrate it have failed.

The precipitin reaction is of value for diagnosis, and works successfully with crude sap.

Introduction

Serological methods have many applications in plant virus work: they give rapid diagnosis, can be used quantitatively, indicate relationships between virus strains and can be used to determine the properties of viruses *in vitro*. So far, however, they have been successfully applied only to viruses transmitted by mechanical methods of inoculation. Several attempts have been made to prepare specific antisera against the sap of plants infected with viruses that have not been transmitted mechanically (Gratia & Manil, 1934; Chester, 1937*b*; Bawden & Kleczkowski, 1944), but all have failed. With such viruses the serological methods would allow a study of *in vitro* properties, which now remain undetermined because of difficulties in transmission.

Reasons for these failures are unknown, though the most likely explanation is that the virus content

in the sap is too low to give a precipitin test. The work described below shows that at least one virus that has not been transmitted mechanically is amenable to serological study, for we have produced antisera that react specifically with the sap of sugar-beet plants infected with sugar-beet yellows virus. We have also used these sera successfully in field diagnosis and to study some of the properties of the antigenic material *in vitro*. It is impossible to be sure that the specific antigen in infected beet is the virus itself. By analogy with other viruses whose serological behaviour has been studied, however, this is most likely (cf. Bawden, 1943), and for convenience the antigen will be called virus.

MATERIAL AND METHODS

The virus used came from a naturally infected sugar beet on Rothamsted farm. *Myzus persicae* was used as a vector, the methods employed being those previously described (Watson, 1940).

Sap was obtained by mincing leaves in a domestic meat mincer and squeezing the mince through bolting silk. When centrifuged for 10 min. at 10,000 rev./min., a layer of fatty material separated at the surface leaving an opalescent supernatant fluid and a pellet consisting of a lower layer of starch and an upper layer of dense green material. Unless otherwise stated, tests were made with the supernatant fluid. It is slightly acid, *p*H 6·5, and the colour varies with the plant; from young healthy plants it is deep green, and from infected plants or maturing leaves it is yellowish green.

Antisera were produced by injecting rabbits intraperitoneally at weekly intervals with 5 ml. of clarified sap. Sugar-beet yellows virus seems less effective as an antigen than other viruses that have been studied. Six injections were sufficient to produce an antiserum that would precipitate with sap from infected plants, but to get a serum with a precipitation titre of 1/200, when tested against sap at 1/4, two courses of injections at an interval of one month were needed. The animals were bled 8–10 days after the last injection.

Precipitin tests were made with antiserum constant at 1/50 and with varying dilutions of the sap. One millilitre of diluted antiserum and sap were mixed in tubes of 7 mm. diameter, which were placed in a water-bath at 40° C., with the fluid column half immersed, so that convection currents ensured continuous mixing.

EXPERIMENTAL RESULTS

Specificity

The serum from rabbits injected with sap from plants infected with beet yellows virus gives a precipitin reaction only with sap from plants infected with this virus. Flocculation begins a few minutes after mixing and a precipitate separates completely within 1 hr. The type of precipitate more nearly resembles that given by tomato bushy-stunt virus than that given by tobacco mosaic virus (Bawden & Pirie, 1938), suggesting that the virus particles are not greatly anisodimensional. No such precipitate is given when sap from infected plants is mixed with saline, with normal rabbit serum, with serum from rabbits injected with healthy beet sap, or with antisera against other viruses. Sap from healthy plants does not seem to contain any appreciable quantity of antigenic material: it gives no specific precipitation with sera from rabbits injected with sap from plants suffering from yellows or with sera from rabbits injected with normal sap. Thus, the serum prepared against sap from infected plants does not need to be absorbed with healthy beet sap before using as a specific antiserum.

Table 1. *Serological activity and aphis transmissions from old and young leaves of plants infected with yellows virus*

	Precipitation after 30 min. Dilution of clarified sap						Aphis transmissions in 20 test plants (3 aphides per plant)
	1/2	1/4	1/8	1/16	1/32	1/64	
Yellowed outer leaves	++++	++++	+++	++	+	−	19
Green inside leaves	++	+	−	−	−	−	2

Sugar-beet sap can precipitate spontaneously when incubated with saline or normal serum. This occurs much more slowly than the specific precipitation, and at 40° C. usually takes several hours. To distinguish between the specific and unspecific precipitation, therefore, control tubes were always set up with the sap mixed with saline or normal rabbit serum.

The precipitin test can be used successfully with crude sap, as described by Chester (1937*a*) for other viruses, either at 40° C. or at room temperature. The precipitin titres are usually about twice as high with crude sap as with clarified sap.

The titres of clarified sap from infected plants grown in the glasshouse have varied from 1/4 to 1/64. The titre varies with sap from different leaves of the same plant, being greater in the yellow older leaves than in the green younger ones. Using three aphides per plant a greater number of infections is also usually obtained from the yellow leaves than from the green ones. This correlation between content of the specific antigen and ease of transmission by *M. persicae* is shown in Table 1.

Properties of the antigen

Resistance to heat

Five-millilitre samples of clarified sap from infected plants were heated in a water-bath for 10 min. at various temperatures. Below 50° C. there was no change in appearance of the sap and its precipitin titre remained unchanged. At 52° C. a coagulum separated leaving a clear yellow supernatant fluid, which did not precipitate with antiserum. Thus, the thermal inactivation point is around 52° C.

To gain further information about the relation between the antigen and the virus, the ability of aphides to transmit the virus from heated leaves was compared with the ability of the sap to react with the antiserum. Detached leaves from infected plants were heated in a water-bath for 10 min. at various temperatures, and were then cut along their midribs. Aphides were fed on one-half of each leaf, while the sap was expressed from the opposite halves and tested with the antiserum. The heated leaves remained sufficiently turgid for the aphides to feed on the individual leaves, and those fed on the best preserved ones gave most infections. On the third day in the test (3) all the infections came from three leaves selected because of their fresher appearance. All the leaves used remained in sufficiently good condition for aphides to feed on them and care was taken to see that only aphides that had fed were used in the transmission tests. The reasons for the fall in transmissibility while serological activity remained unimpaired were unknown. It is, of course, possible that some changes within the leaves, which had nothing to do with the virus, militated against transmission by aphides, but other viruses are known, e.g. tomato bushy-stunt virus, which lose infectivity but not serological activity when stored.

Effect of acid, alkali and trypsin

Clarified sap was adjusted to various *p*H values, measured by a glass electrode, by the addition of 0·1 *N* hydrochloric acid or 0·1 *N* sodium hydroxide. After 30 min. the *p*H was brought back to 6·5, and the fluids left for 1 hr. with occasional stirring; then they were centrifuged and the supernatants tested with the antiserum.

In the fluids exposed to *p*H 5·0 and 4·5 irreversible precipitates formed. After exposure to *p*H 4·5, the supernatant fluid was water clear and yellow and did not react with the antiserum. After exposure to *p*H 5·0 the supernatant fluid was slightly opalescent and gave a precipitin titre of 1/8 compared with 1/64 for the control. Addition of alkali to *p*H 9·0 caused no change in the sap and had no effect on the serological activity.

Clarified sap became slightly less opalescent when incubated at *p*H 7·5 and 37° C. for 3 hr. with 0·5 % commercial trypsin, but its serological activity was unaffected.

Freezing and dialysis

Freezing clarified sap and thawing after 24 hr. did not affect the serological activity or cause any visible change in the appearance of the sap. Dialysis of the clarified sap in a cellophane sac against distilled

TABLE 2. *Effect of heating whole detached leaves on serological activity and transmissibility of the virus by the vectors. Tested on two occasions,* (1) *and* (2)

Whole leaves heated for 10 min. at		Precipitation after 30 min. Dilution of clarified sap 1/4	1/8	1/16	1/32	1/64	Aphis transmissions in 20 plants (3 aphides per plant)
Unheated	(1)	++++	++++	+++	++	+	12
	(2)	++++	+++	++	+	–	9
47·5° C.	(1)	++++	+++	++	+	–	7
	(2)	+++	++	+	–	–	12
50° C.	(1)	+++	++	+	–	–	7
	(2)	++	++	+	–	–	5
52·5° C.	(1)	++	+	–	–	–	1
	(2)	+	–	–	–	–	0
54° C.	(1)	+	–	–	–	–	1
	(2)	–	–	–	–	–	0

satisfactorily. It will be seen from Table 2 that the ability of the aphides to transmit the disease from the heated leaves fell with the serological activity.

Resistance to ageing

Clarified sap expressed from infected plants ceased to precipitate with the antiserum, after it had been kept for 3 days at room temperature, but in detached leaves the serological activity persisted longer. Table 3 shows the results of tests in which detached leaves wrapped in moist blotting paper were stored in closed tins at room temperature for various periods. The leaves were cut in halves, one lot being used for transmission tests by aphides and the other for serological tests. It will be seen that the precipitin titre of the sap remained constant for the 6 days, whereas the ability of aphides to transmit the virus from the leaves fell off slowly during the first 3 days and then more rapidly. All the leaves appeared to be in perfect condition until the third day of storage when some showed signs of deterioration. Details were kept of the performance of aphides fed

water for 3 hr. at room temperature also had no effect on the serological activity or the appearance of the sap, but when dialysed sap was frozen and thawed it failed to precipitate with antiserum, although it did not change in appearance. The addition of 1 % sodium chloride or 2 % sucrose to the dialysed sap before freezing prevented the destruction of the antigen. In this property sugar-beet yellows virus resembles tomato bushy-stunt virus (Bawden & Pirie, 1943).

Attempts to purify and concentrate the virus

Filtration of the clarified sap through a layer of celite about ½ cm. thick considerably decreased its opalescence, but it also reduced the precipitin titre from 1/16 to 1/4. Filtration through Chamberland candle L1 gave an almost clear fluid and reduced the precipitin titre to 1/2, and filtration through L3 candle gave a water clear yellow fluid which did not react with the antiserum.

20,000 rev./min., about half the virus sedimented and, at 40,000 rev./min., the whole of it. The sediment was dark green and closely resembled the precipitate produced by ammonium sulphate; it redissolved completely in water. When the sediment obtained by centrifugation at 40,000 rev./min. was redissolved and centrifuged a second time at 20,000 rev./min., the whole of the antigen was sedimented; this suggests that centrifugation leads to aggregation of either the virus or of some sedimentable plant constituents.

All our attempts to concentrate the virus have failed. When either the precipitate with ammonium sulphate or the material sedimented by high-speed centrifugation from a large volume of sap was resuspended in a small volume of water, only a part dissolved giving fluids resembling the original sap in appearance and in serological activity; a second extraction of the precipitate also gave a fluid resembling the first extract.

TABLE 3. *Effect of keeping on serological activity and transmissibility of yellows virus in detached leaves. Tests repeated on three occasions,* (1), (2) *and* (3)

Days kept		Precipitation after 30 min. Dilution of clarified sap 1/4	1/8	1/16	1/32	Aphis transmissions in 20 plants (3 aphides per plant)
0	(1)	++++	+++	++	+	9
	(2)	+	+++	++	+	10
	(3)	+++	+++	++	+	14
1	(1)	++++	+++	++	+	7
	(2)	++	+++	+	−	9
2	(1)	++++	+++	++	+	5
	(2)	+	+++	++	+	5
	(3)	+++	+++	++	+	9
3	(1)	++++	+++	++	+	1
	(2)	++	+++	++	+	4
	(3)	+++	+++	++	+	10
4	(2)	+	++	+++	+	1
5	(3)	+++	+++	++	+	3
6	(3)	+++	+++	++	+	1

Like other viruses, beet yellows virus can be precipitated with ammonium sulphate or sedimented by high speed centrifugation. Part of the virus in clarified sap was precipitated by 1/4 saturated, and the whole by 1/3 saturated, ammonium sulphate. These treatments gave a bulky, dark precipitate that redissolved almost completely in a volume of water equal to the original volume of sap. The fluid thus obtained after precipitation with 1/3 saturated ammonium sulphate did not differ in appearance from the original sap and gave the same precipitin titre. Repeated precipitations with ammonium sulphate gave only a slight decrease in the precipitin titre, but gave no useful fractionation, for all the materials precipitating with the virus also redissolved readily. Sap previously frozen or incubated with trypsin gave similar results.

When clarified sap was centrifuged for 1 hr. at

DISCUSSION

The antigen whose properties we have studied is clearly a product specific to virus-infected plants, but it has not been possible to prove that it is the virus itself. Specific precipitation has been obtained only with sap from infected beet: yellowed leaves from plants suffering from downy mildew and various mineral deficiencies all failed to react with the antiserum. The antigen is also specific to plants infected with yellows virus as no reaction is obtained with sap from plants suffering from mosaic. There is also correlation between the antigen content of leaves and the ability of *M. persicae* to become infective when feeding on them. The strongest reason for regarding the antigen as the virus itself, however, is its similar behaviour to other viruses whose properties have been studied in detail.

The properties of the beet sap are unfavourable

for work with the virus *in vitro*, for it seems that normal beet sap contains much material that resembles the virus in its precipitability and stability. This fact probably prevented successful attempts to fractionate and concentrate virus preparations. Plants which have been used as hosts of other viruses have also been found to contain proteins that sediment in the high-speed centrifuge and precipitate with 1/4 saturated ammonium sulphate solution, but these constituents have usually been readily separable from the viruses because of their greater sensitivity to heating, freezing, *p*H changes or proteolytic enzymes. In all these properties, however, except for freezing, towards which the virus is more sensitive than the normal beet constituents, sugar-beet yellows virus resembles normal constituents of beet sap. Indeed, the one striking difference we have found between the two is the antigenicity of the virus. It seems that the methods that have been successfully used to purify viruses attacking solanaceous plants may not be equally valuable for other hosts. That beet is not remarkable in this respect is shown by the fact that Loring *et al.* (1938) failed to separate pea mosaic virus from normal constituents of bean sap by high-speed centrifugation.

Whether or not the antigen is the virus, the precipitin test is specific and can be used for diagnostic purposes. Many different causes can lead to the yellowing of beet leaves, and it is useful to have a rapid test for yellows virus. The use of crude sap and the specific antiserum provides this and the test often works well on plants from the field. However, the position is not perfectly straightforward, as there are factors that complicate the precipitin test. Using antiserum at 1/50, the precipitin titres of saps from plants infected in the field have varied from 1/64 downwards, and it is obvious that the virus content of some plants may be insufficient to give a precipitin reaction. Also sap from some plants failed to react with the antiserum at 1/50, although it reacted when the antiserum was used at a higher concentration. This was not because the virus content was so high as to prevent precipitation by antigen excess, for the precipitin titres were not high and no reaction was obtained with the antiserum at 1/50 when the sap was greatly diluted. The precipitation with more concentrated antiserum was restricted to a narrower zone of antigen/antibody ratios, and the results suggest that the saps contained material that interfered with serological precipitin reaction.* No such effect has been noticed with sap from infected plants grown in the glasshouse. It is also possible that sugar-beet yellows can be caused by serologically unrelated viruses, as is tobacco necrosis (Bawden, 1941; Bawden & Pirie, 1942), although we have no evidence of this and we have tested infected plants from many different districts. Therefore at present, although a positive precipitin reaction can be accepted as proof of the presence of sugar-beet yellows virus, a negative test does not exclude it.

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REFERENCES

Bawden, F. C. (1941). The serological reactions of viruses causing tobacco necrosis. *Brit. J. exp. Path.* **22**, 59.

—— (1943). *Plant Viruses and Virus Diseases.* Waltham, Mass.: Chronica Botanica Co.

Bawden, F. C. & Kleczkowski, A. (1944). Protein precipitation and virus inactivation by extracts of strawberry plants. *J. Pomol.* (in the Press).

Bawden, F. C. & Pirie, N. W. (1938). Crystalline preparations of tomato bushy-stunt virus. *Brit. J. exp. Path.* **19**, 251.

—— —— (1942). A preliminary description of preparations of some viruses causing tobacco necrosis. *Brit. J. exp. Path.* **23**, 314.

—— —— (1943). The inactivation of tomato bushy-stunt virus by heating and freezing. *Biochem. J.* **37**, 70.

Chester, K. S. (1937*a*). A simple and rapid method for identifying plant viruses in the field. *Phytopathology*, **27**, 722.

—— (1937*b*). Serological studies of plant viruses. *Phytopathology*, **27**, 903.

Gratia, A. & Manil, P. (1934). De quelques échecs de la méthode sérologique appliquée aux virus de plantes. *C.R. Soc. Biol., Paris*, **118**, 379.

Loring, H. S., Osborn, H. T. & Wyckoff, W. G. (1938). Ultracentrifugal isolation of high molecular weight proteins from broad bean and pea plants. *Proc. Soc. exp. Biol., N.Y.*, **38**, 239.

Watson, M. A. (1940). Studies on the transmission of sugar-beet yellows virus by the aphis *Myzus persicae* (Sulz.). *Proc. Roy. Soc.* B, **128**, 535.

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* *Note added in proof.* Further work has increased the probability that the failure to precipitate was due to the presence of an inhibitor in the sap; also material which inhibited serological precipitation of tomato bushy-stunt virus has been isolated by Bawden & Pirie (*Brit. J. Exp. Path.* 1944, **25**).

Stability of the microbial populations of the caecum of guinea-pigs and rabbits

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The microbial population of the caecum of rabbits and guinea-pigs, respectively, normally exhibits distinctive features which can be tabulated precisely, and which persist when the animals are kept in the same cage and fed on the same diet. A pronounced resistance, therefore, opposes cross-infection and tends to secure the stability of the population whose characteristics afford useful criteria for the microbiological control of *in vitro* incubation experiments.

Introduction

Direct microscopic observation demonstrates that the microbial population of the rumen or caecum may exhibit distinctive characteristics in different herbivorous species. Such populations can include a protozoan microfauna of ciliates and flagellates, an iodophile microflora, an aniodophile microflora.

The iodophile microflora embraces macroiodophile and microiodophile species. Also a fixed iodophile population attached to plant material may be distinguished from a free iodophile population suspended in the liquid of the rumen or caecum.

Questions arise regarding the extent to which the observed differences in the composition of one or more of these groups is influenced by the composition of the diet and by the natural or artificial isolation of different herbivorous species one from another. Investigation of the rumen population of the ox (Baker, 1943) has shown that the effect of diet may be quantitative rather than qualitative over a wide range of foodstuffs. It remains, therefore, to consider the effects of isolation. This can be investigated by comparing the microbial populations of animals of different species kept in the same cage and fed on the same diet with those of the same animals kept apart. Guinea-pigs and rabbits were employed, since it had been determined (Baker, 1933; Baker & Martin, 1937 *a*, *b*) that the difference between the respective caecal populations is here pronounced and persistent. Moreover, since the rabbit is habitually coprophagous, good conditions for faecal infection were secured. An examination of the gastric and rectal contents was also made. In order to elucidate certain observations it was found necessary to determine the characteristics of the caecal population of rabbits of different ages.

Methods

In experiments of this kind it is imperative to formalize the gut contents of several animals simultaneously and immediately after death. This restricts the number of animals that can be dealt with at one time by an investigator. Small sets were therefore employed and the experiment was repeated three times. Each set comprised two adult guinea-pigs, one adult rabbit and a rabbit of 1 month. The animals were provided with hay and fed a mixture of carrots, Brussels sprouts tops, cooked and uncooked potato, and tea leaves: 24 hr. previous to slaughtering the diet consisted chiefly of uncooked potato. Experience has shown that this dietary secures the development of a large iodophile population. The animals were kept together for 14 days and were feeding up to 2 hr. *ante mortem*. The methods employed in the direct microscopical examination of gut contents have been described by Baker & Martin (1939).

Observations

The elucidation of the results of these experiments presupposes some acquaintance with the normal caecal populations of the animals concerned. Since details may be found in the papers already cited description is abridged. The micro-organisms referred to are those indigenous to the caecum of adult animals.

Protozoan population

Guinea-pigs. Entodiniomorpha are absent. A flagellate population is always present, the prevailing genus being commonly *Trichomonas*.

Rabbits. Entodiniomorpha absent. Flagellates population restricted or absent.

Iodophile population

(*a*) *Free iodophile population*

(1) *Microiodophile species.* Since differential morphological criteria are here lacking, comparisons are precluded. The population in both rabbits and guinea-pigs comprises innumerable cocci, small rods, and vibrios.

(2) *Macroiodophile species. Guinea-pig.* The following types can readily be distinguished: (i) Giant amylococcus. Chains of 4–8 cells are frequent. (ii) A giant navicular bacillus of the amylobacter

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type, which will be referred to as *Amylobacter* (*a*). (iii) A colourless oscillarian, *Oscillospira guilliermondi* (Chaton & Pérard). (iv) A large ellipsoidal organism of uncertain taxonomic position with elongated thick-walled spores embedded in strongly iodophile plasma. It will be referred to as 'ellipsoidal type'. *Rabbit.* (i) Giant amylococcus. The amylococci of rabbits usually assume the diplococcus form. Chains of more than four units are infrequent. (ii) A giant *Amylobacter* type similar to (ii) above but having rounded in place of pointed ends. It will be referred to as *Amylobacter* (*b*).

(*b*) *Fixed iodophile population*

The types concerned with the decomposition of cellulosic structures are similar in both animals. Whereas, however, microiodophile cocci are involved in the disintegration of starch grains in rabbits, in guinea-pigs vibrios predominate.

Aniodophile population

Fusobacteria, i.e. elongated non-septate sinuous threads with pointed ends and containing metachromatic granules.

Spirochaetes of familiar buccal-intestinal types.

Selenomonas. An organism of uncertain taxonomic position characterized by curved shape and mediolateral insertion of a single flagellum on the concave surface.

Giant spirilla with tapering ends and bipolar monotrich flagellation.

Rabbit. The sole readily identifiable species is an elongated ellipsoidal yeast, which can also be observed in the gastric contents. In the latter locality cells in process of budding are frequently encountered. The organism can only be ranked amongst aniodophile species if the term iodophile is confined to types containing a blue-reacting polysaccharide. It contains abundant red-reacting (iodine) glycogen reserves. The absence or extreme infrequence of spirochaetes and fusobacteria from the caecum of rabbits has already been recorded (Baker & Martin, 1937*a*) and is noteworthy in view of their widespread distribution in vertebrates.

The aforementioned data may be tabulated as follows for purposes of comparison:

	Trichomonas	*Amylococcus*	*Amylobacter* (*a*)	*Amylobacter* (*b*)	*Oscillospira*	'Ellipsoidal type'	*Fusobacterium*	*Spirochaeta*	*Spirillum*	*Selenomonas*	Yeast
Guinea-pig	+	+	+	−	+	+	+	+	+	+	−
Rabbit	−	+	−	+	−	−	−	−	−	−	+

Thus, *Amylobacter* (*b*) and yeast are normally absent from adult guinea-pigs, whilst *Trichomonas*, *Amylobacter* (*a*), *Oscillospira*, 'ellipsoidal type', *Fusobacterium*, *Spirochaeta*, *Spirillum* and *Selenomonas* are absent from rabbits.

Examination of gastric, caecal and rectal contents of animals kept in same cage

Adult rabbits

Caecal contents. An abundant iodophile microflora was present. Bacterial disintegration of starch granules was conspicuous, the granules being entirely surrounded by iodophile micro-organisms. The predominant species were microiodophile cocci. *Trichomonas*, *Amylobacter* (*a*), *Oscillospira*, 'ellipsoidal type', *Fusobacterium*, *Spirochaeta*, *Spirillum* and *Selenomonas* were absent. It is clear, therefore, that no infection with guinea-pig species had occurred.

Gastric contents. Starch granules were present in abundance, but showed no signs of either enzymatic or bacterial decomposition. Very few iodophile micro-organisms were observed. Yeast was present in great numbers. No infection with guinea-pig species was demonstrable.

Rectal contents. The reduction in numbers of the iodophile population was conspicuous. Yeast still persisted, but the cells showed evidence of autolysis. No budding forms were observed.

Rabbits of 1 month

Caecal contents. The iodophile microflora was similar to that of adults, no infection with guinea-pig species being demonstrable. Spirochaetes and fusobacteria were, however, present in large numbers.

Evidently two hypotheses could be advanced to account for this fact. Thus, either (*a*) infection from guinea-pigs had occurred, or (*b*) spirochaetes and fusobacteria, though absent from adults, are present in young rabbits. An examination was, therefore, made of caecal contents of rabbits of 3 weeks and 7 days old respectively. The former had been nibbling at vegetable material for a period of about one week, the latter were suckling. Both had been kept in strict isolation from guinea-pigs.

Rabbits of 3 weeks (isolated)

Caecal contents. An abundant iodophile microflora of the normal type was present. Yeast, however, was not observed. Spirochaetes and fusobacteria were present in large numbers.

Rabbits of 7 days (isolated)

Caecal contents. The iodophile population showed only a restricted development and was confined to microiodophile species. No yeast was present.

Spirochaetes and fusobacteria were present in abundance.

From these facts it became clear that spirochaetes and fusobacteria are normally present in young rabbits and sucklings and that these, therefore, do not afford suitable material for cross-infection experiments.

Adult guinea-pigs

Caecal contents. An extremely dense microbial population was established. All the types tabulated were present. *Oscillospira* was observed in exceptionally large numbers, as were also both spirochaetes and fusobacteria. Evidently, therefore, the conditions prevailing in the experiment were such as to afford optimal opportunity for the infection of rabbits with characteristic guinea-pig species. Active bacterial disintegration of starch granules by vibrios was observed. Neither *Amylobacter* (*b*) nor yeast was observed. No infection, therefore, with rabbit micro-organisms had occurred.

Gastric contents. Partial enzymatic digestion of starch was indicated by the change and decrease in iodine reaction of some of the granules present. The granules were entirely free from attached bacteria. In the surrounding liquid, however, amylococci were present in considerable numbers though no other macroiodophile species were detected. No infection with rabbit micro-organisms, including yeast, had occurred.

Rectal contents. A marked decrease in the iodophile population was apparent. Neither *Trichomonas* nor *Oscillospira* was observed. Starch had entirely disappeared.

CONCLUSION

The conclusions to be drawn from the foregoing results corroborate and extend those previously advanced. Thus, the customary reduction of the caecal population during passage through the large gut is again confirmed. In addition, it is demonstrated that certain representatives of the population may establish themselves in the stomach of the same animal, notably a species of yeast in rabbits, and *Amylococcus* in guinea-pigs. This fact is significant, since it indicates that the distribution and density attained at various levels throughout the alimentary tract by particular members of the mixed microbial population is determined (in each herbivorous species) by a complex of regional factors. On this view the tendencies towards a focal allocation of the population to the caecum or rumen, respectively, represent extreme instances between which we could expect to discover intermediaries wherever, as is the case in certain rodents (Wiedersheim, 1886), both gastric and colonic diverticula are developed comparably. Investigations in progress at this laboratory on the hamster lend provisional support to such an assumption.

No less apparent is the fact that the age of the animal as well as the regional characteristics of the gut diverticula exert an influence on the composition of the microbial population. Thus, the features witnessed in the adult represent the climax phase of a microbial succession initiated in the suckling. One of the factors, consequently, that may in part determine the observed differences between the adult microbial populations of various herbivora is the actual extent to which the micro-organisms present in the initial endure to and are incorporated in the terminal association.

Turning now to the problem of isolation, the results obtained demonstrate that a considerable resistance opposes cross infection, since the representative populations of adult guinea-pigs and rabbits retain their specific characteristics despite identity of diet and close association of the animals. To suppose that this resistance is absolute and that cross infection is, under any circumstance, unconditionally excluded would be premature. None the less, it appears legitimate to conclude that, under normal circumstances, it is of such an order as to secure the stability of the microbial population, whose features accordingly afford a genuine basis both for comparative studies and for the control of *in vitro* incubations.

REFERENCES

BAKER, F. (1933). Studies in the microbiology of organisms associated with the disintegration of vegetable remains. *Zbl. Bakt.* Abt. II, **88**, 17.

—— (1943). Direct microscopical observations upon the rumen population of the ox. I. Qualitative characteristics of the rumen population. *Ann. appl. Biol.* **30**, 230.

BAKER, F. & MARTIN, R. (1937*a*). Some observations on the iodophile microflora of the caecum of the rabbit. *Zbl. Bakt.* Abt. II, **96**, 18.

BAKER & MARTIN (1937*b*). Observations upon the disintegration of cell-wall substances in the caecum of the guinea-pig. *Zbl. Bakt.* Abt. II, **97**, 201.

—— —— (1939). Studies in the microbiology of the caecum of the horse. *Zbl. Bakt.* Abt. II, **99**, 400.

WIEDERSHEIM, R. (1886). *Lehrbuch der vergleichenden Anatomie der Wirbelthiere.* Jena.

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Chafer damage to grassland in north Wales in 1942–1943 by *Phyllopertha horticola* L. and *Hoplia philanthus* Fuess.

I. Notes on population, life history and morphology

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(With 12 Text-figures)

An account is given of a severe attack to grassland by chafer-beetle larvae in some north Wales valleys in the years 1942–3. The species concerned were *Phyllopertha horticola* L. and *Hoplia philanthus* Fuess. Estimations of the populations of both species were made and notes are given on the biology and comparative morphology of their immature stages. Both species have a one-year life cycle; all stages of *Hoplia philanthus* occur somewhat later than *Phyllopertha horticola*.

Introduction

The extent to which grassland in some north Wales valleys was being attacked by chafer larvae in the autumn of 1942 made an investigation into the problem an urgent necessity. Detailed observations were commenced in November of that year, and by December it was found that, in addition to the larvae of *Phyllopertha horticola* L., larvae of *Hoplia philanthus* Fuess. were in some areas responsible for severe damage. The co-operation of the Department of Agricultural Botany was sought in studying the effect of the injury to various grasses and for work on control measures. It is intended to follow the present paper with one on ecology and control by R. E. Hughes and D. W. Parry.

History of previous attacks

Curtis (1883) records what is probably *P. horticola* under both the names *Anisoplia agricola* L. and *A. horticola* L.; quoting a Mr Dillwyn he reports adults in immense numbers at Swansea in 1814. Ormerod (1893) records adults flying in thousands in Cheshire in 1885. The years 1891–5 saw what were probably the most widespread and severe attacks until those now being described. The affected areas included localities in Monmouthshire, Kent, Surrey, Hampshire and central Wales. Adults are recorded as swarming in large numbers and damaging 'leafage', etc., and larvae ravaging grassland. Since then there have been numerous records of the occurrence of adults and larvae, but no very widespread and severe attack has been reported.

Less widespread but fairly severe attacks were noted by Warburton (1909, 1911). These extended to Dolwyddelen in north Wales where severe damage is remembered about this time by Dr Bryner Jones and Prof. R. G. White. Walton (1928) records larvae as being very numerous in north Wales mountain pasture in 1915 and in root crops at Clynnog (south Caernarvonshire) in 1921. Attacks to grassland in Yorks and Bucks and to barley in Cheshire occurred in 1919–20: the Yorkshire attack was described by Taylor & Thompson (1928).

The Ministry of Agriculture's Reports on Insect Pests of Crops for 1928–31 (1933) and 1932–4 (1936) contain a number of records of damage by larvae of *P. horticola*. There was a severe attack to a pasture in Notts in 1931. In 1933 infestations above normal occurred in the West Midland, South-eastern, South and Western Agricultural Advisory Provinces of England, but numbers were below normal in the following year. The year 1935 saw the beginning of the severe attacks experienced over a number of years in south and mid-Wales and in Westmorland. With only a slight abatement of the damage these attacks appear to have persisted until 1943. From 1935 to 1943 sporadic attacks have also occurred in Notts, Cheshire, Lancs, Dorset, Essex, Surrey, Somerset, Glos, Devon, Berks and Shropshire.

In the summer of 1938 adults were reported as swarming in the Conway Valley and damaging apple fruitlets. In the following November a small hillside pasture was severely damaged at Llangollen. In November 1941 a case of moderate damage to hill grazing land at Capel Curig was examined and this was said to be the second year of the attack. By the autumn of 1942 the attack in north Wales had become widespread and severe, and in December larvae of *H. philanthus* were first found in the Beddgelert district. Fidler (1936*b*) does not deal with either *P. horticola* or *H. philanthus*, but his records of damage by chafer larvae probably include damage by the former.

The 1942–3 attack in north Wales

Fig. 1 shows the distribution of damage to grassland in north Wales in 1942–3. The damage by *P. horticola* first became noticeable in September when an examination of infested fields showed that large numbers of 3rd instar larvae were present. In the worst areas most of the grasses were killed and the turf was scratched and further loosened by birds until large areas were laid bare. The birds chiefly concerned were rooks, carrion crows and jackdaws. Starlings have also been seen following the plough and feeding on 2nd instar *H. philanthus* larvae. Oyster-catchers

and curlews were occasionally seen feeding on chafer larvae and hens also searched affected fields in the neighbourhood of farm-houses.

The damage by *H. philanthus* larvae was identical with that of *P. horticola*. The affected areas extended during the winter, but by late spring both species appeared to have finished feeding and no further damage occurred until the following late summer.

The areas affected by *P. horticola* were largely confined to the wooded river valleys. Damage was bordering the Afon Glaslyn as far as Beddgelert. It occurred in small numbers at Llanfaglan near Caernarvon where it was the only species present. One *H. philanthus* larva was found near Dolwyddelen.

Only very occasional larvae of *Melolontha* sp., *Amphimallus solstitialis* and *Serica brunnea* were seen.

ESTIMATION OF POPULATIONS

In the course of the work on the estimation of wireworm populations in north Wales previous to 1941,

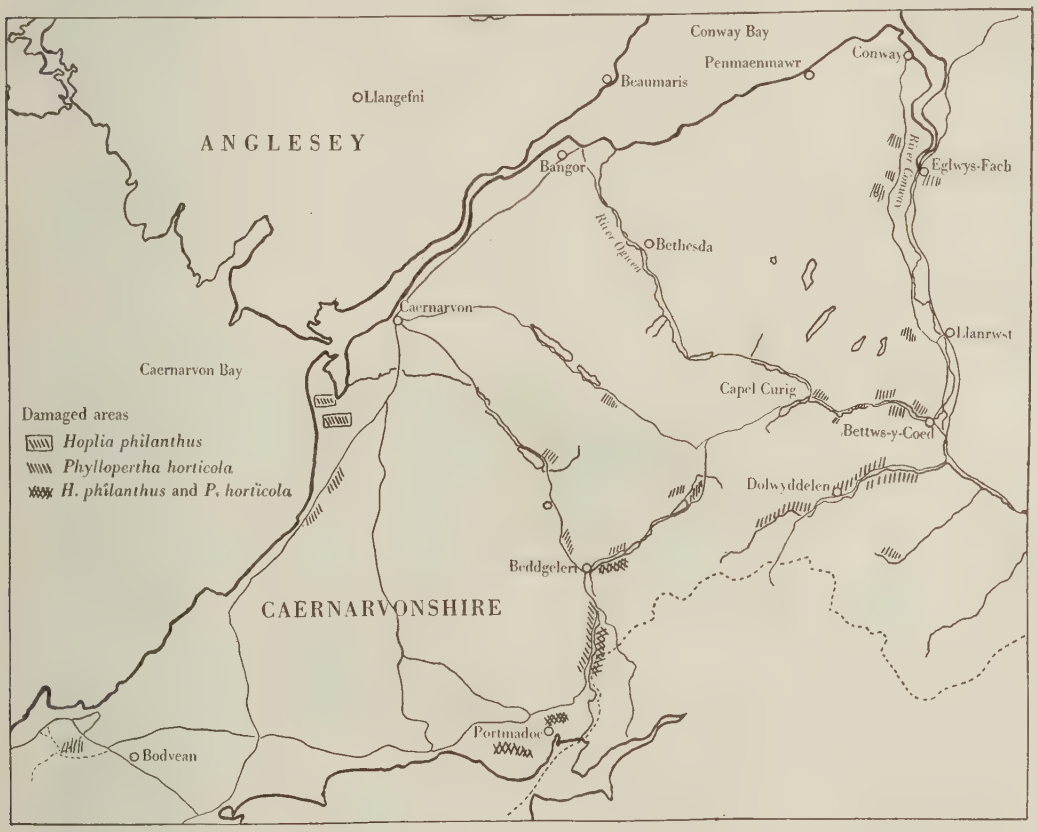

Fig. 1. Map of north Wales showing distribution of damage by larvae of *Phyllopertha horticola* L. and *Hoplia philanthus* Fuess. in 1942 and 1943.

mainly concentrated on the level near the rivers and on the adjacent slopes, but it sometimes extended to a height of about 900 ft. Both hill grazing land and hay fields were equally affected, but in most cases only fields of poor grasses, obviously in poor heart, were seriously attacked.

H. philanthus was dominant and present in large numbers only in the Portmadoc district. There the affected fields were very sandy and almost at sea level, much of the land having been reclaimed. It was also present in smaller numbers farther inland in the fields

chafer larvae had sometimes been present in the soil samples. Other workers have reported chafer populations found during the course of wireworm survey work. For the chafer work two different methods were tried. A number of cubic foot samples of soil in affected fields were examined, and the wireworm estimation method as described by Finney (1941) was tested. For *P. horticola* this method gave the results recorded in Table 1.

Of 163 larvae found in 18 cu. ft. of soil only two occurred at a greater depth than 6 in., and it was

therefore decided that if the wireworm sampling method gave reasonably accurate estimations it could safely be used since very few larvae would occur below the depth of the borer. In February 1943 the figures from sampling a number of fields were submitted to Mr D. J. Finney of Rothamsted Experimental Station who reported provisionally that the variability within the field is of the same order of magnitude as for wireworms. Twelve fields randomly sampled gave *P. horticola* populations varying from 0 to 950,000/acre. The results of fields selectively sampled are shown in Table 2. Damaged areas, i.e. areas badly scratched up by birds, and undamaged areas were sampled separately in order to determine whether the birds concentrated on the areas of highest population.

Table 2 shows much higher populations in the damaged areas in every field except one. Field 20 sampled at random showed no larvae in twenty samples. Field 23 was adjacent to a field with severe damage; the damage was negligible and yet it gave a population of 400,000/acre. This field was a better pasture than its neighbours, but field 19 shows that undamaged parts of a field can harbour populations of approximately 600,000/acre.

TABLE 1. *The number of larvae of* Phyllopertha horticola *found at different depths in three separate cubic foot samples per field*

Field no. ...	1			2			3			4			5			6		
Date sampled ...	24. xi. 42			24. xi. 42			26. xi. 42			4. xii. 42			1. xii. 42			21. xii. 42		
Depth (in.)	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
0– 3	2	0	2	5	4	3	14	5	14	0	2	7	77	22	23	45	10	16
3– 6	6	2	5	0	1	0	1	5	33	0	0	1	0	1	0	3	0	11
6– 9	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
9–12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 2. *Chafer populations in north Wales in thousands/acre. Fields selectively sampled*

Field no.	Damaged area		Undamaged area	
	P. horticola	*H. philanthus*	*P. horticola*	*H. philanthus*
15	800	—	150	—
16	1700	—	100	—
17	800	—	50	—
18	800	—	550	—
19	1050	—	600	—
20	800	50	50	—
21	1850	—	50	—
22	1400	—	250	—
23	No apparent damage		400	—
24	775	225	200	50
25	175	175	—	50
26	450	1000	—	300
27	650	300	—	—
28	250	—	50	—
29	100	150	150	200

NOTES ON LIFE HISTORIES

P. horticola. A full account of the biology and morphology of *P. horticola* has been given by Rittershaus (1927). The adults of this species swarm towards the end of May and during June and early July, and they feed on a variety of plants including forest and fruit trees. In north Wales the earliest record of *P. horticola* adults is 21 May, when three specimens were seen by a Beddgelert farmer on hillside bracken. On 24 May, nine adults were collected in this district and pupae and adults were taken from the soil; none remained in the larval stage. On 31 May newly emerged adults were seen swarming on the wing, low over the ground in other Beddgelert fields where no emerged adults were to be found on 24 May. It was a warm sunny day and immense numbers were in flight over a large area; they were most dense within 9 in. of the ground, but a number were flying much higher: twelve to fifteen would alight on a rucksack within the space of a few minutes. Subsequently they were readily to be found on bracken and ragwort, and on fruit and woodland trees. At Beddgelert they damaged young apple fruits, and at Dolwyddelen broad beans in a garden were injured.

The egg stage was missed, but 1st instar larvae were found in very large numbers on fields at Dolwyddelen on 22 July. Rittershaus (1927) states that eggs are laid towards the end of June and that they take 15–20 days to hatch. Harper Gray (unpublished) found eggs and newly hatched larvae on 28 June 1940.

By 18 Aug. all the larvae found were in the 2nd instar and all were in the 3rd instar by 8 Sept. On the latter date, three fields sampled at Dolwyddelen gave populations of 2,750,000, 2,200,000 and 150,000/acre respectively. Even as early as July the birds had detected the presence of the larvae and had scratched up small bits of turf. By 8 Sept. there was considerable damage in the fields with the high populations, but none in those with low populations.

Occasional depth sampling during the winter showed that the larvae remained in the top 6 in. of turf, but this may have been the result of the very mild winter. In the Ministry of Agriculture monthly summaries (unpublished) Harper Gray in 1938 reported 'grubs numerous at a depth of 4–6 in. on hill

grassland in Westmorland at the end of March', and populations reported by other workers are: Edwards, up to 750,000/acre in Glamorgan in 1942; Jenkins, 'On 35 fields in Merionethshire, almost entirely confined to the coastal districts, the average larval population was 143,000/acre, the two highest records being 1,008,000 and 964,000/acre.'

Larvae brought to Bangor had all pupated out of doors on the roof by 6 Mar. 1943. Larvae were near to pupation in the field on 28 Apr., and on 6 May at Dolwyddelen pupae were numerous in the field and all larvae were in the prepupal condition. Adults were seen from 21 May onwards. On 24 and 25 May in thirty 4 in. core samples from Beddgelert there were two larvae, eight pupae and seventeen adults. In a field at Portmadoc forty-six 3rd instar larvae of *P. horticola* were found in a 6 in. square of turf.

H. philanthus. Fowler (1888–91) and Fowler & Donisthorpe (1913), referring to the distribution of *H. philanthus*, give a limited list of localities in England and mention Swansea, Barmouth and Bog of Arthog in Wales as well as one record from Scotland. Fowler also quotes 'abundant on our western border and about Lanercost (Bold)'. The habitat is given as 'on flowering shrubs and plants; local but usually common where it occurs'. Three more recent reports are included in the Ministry of Agriculture monthly summaries (unpublished): in 1937, in Glamorganshire, Edwards found adults in numbers emerging from a bowling green and in 1942, also in Glamorganshire, the beetles caused injury to the foliage of fruit trees; in 1939, Fox Wilson reported 'adults swarming on a lawn at Ascot, Berks.'

When first found in December 1942 all the larvae taken were in the 2nd instar. 3rd instar larvae were first found on 19 Jan.; by 18 Feb. at Portmadoc more than half the larvae found were in the 3rd instar and at Beddgelert on 2 Mar., of twenty-one larvae found, eleven were in the 3rd instar, eight in the 2nd and two in the 1st instar. Most of the larvae had pupated by 1 June, and although careful watch was kept for the adults in the districts where pupae were abundant, it was not until 29 June that they were found swarming on young oaks and sallows in the Beddgelert district. It was a very hot sunny day following 3 days of fine warm weather. Subsequently the weather deteriorated, but on the occasional good days no adults were taken again until 16 July when they occurred in large numbers on the flower heads of bent and Yorkshire fog in a field at Portmadoc where previously the larvae had done damage. No adults were found after this date, and it appears that swarming occurs only on very warm days after a few days of hot sunny weather. At other times they probably return to the soil because beating, sweeping and thorough searching of plants did not produce a single specimen. A number of beetles were taken in copula on both dates, and on 16 July three females were caged on a very small piece of sandy turf. By the following day the beetles had all burrowed into the soil, and on 20 July eggs were found at a depth of 2–4 in. by which time the adults had died. On 4 Aug. three more eggs and two 2nd instar larvae were found in the turf which had not previously been disturbed. The eggs were removed to a moist dish; two had hatched by 18 Aug. and the remaining one by 26 Aug. Twenty-eight larvae, all in the 1st instar, were found in the field at Portmadoc on 12 Aug. On 11 Sept. only 2nd instar larvae were present, and they were still in the 2nd instar on 31 Dec. 1943. The duration of the egg stage and larval instars appears to be very variable, and although the great majority complete their life cycle in one year, a few probably take longer.

In addition to the area affected by *H. philanthus* in Caernarvonshire, the species was found (with *P. horticola* larvae) in two fields near Maentwrog (Merionethshire) near the estuary of the Dwyrhyd.

NOTES ON THE MORPHOLOGY OF THE LARVAE AND PUPAE

The morphology of *P. horticola* having been fully described by Rittershaus (1927) only a few details are given for comparison with *H. philanthus*.

P. horticola and *H. philanthus* belong to the subfamilies Rutelinae and Melolonthinae respectively, and although it seems certain that larvae of the latter have been mistaken for *P. horticola* they can easily be distinguished. Both are typical white fleshy chafer larvae, popularly known as white grubs. The dorsal surfaces of abdominal segments 2–7 in both species are covered with short spine-like hairs (Fig. 2 A, B), but those of *H. philanthus* are thicker, stronger and more heavily chitinized and are slightly raised on chitinous bases. They are also more numerous than on *P. horticola*, and being rust coloured they give a rusty appearance to the humped part of the back and even in the field this character makes the larvae easily distinguishable.

The ventral surface (raster) of the last abdominal segment in *P. horticola* bears two longitudinal rows of numerous short spinules and a few longer slightly recurved spines; in *H. philanthus* it bears irregularly dispersed strong spines with backwardly projecting apices (Fig. 2 C).

The *antennae* of *P. horticola* and *H. philanthus* consist of four joints, the penultimate joint in each case being produced on the ventral surface. This prolongation is more prominent in *H. philanthus* and bears a sensorium on its ventral surface, the apex itself being tipped with sensillae. The terminal joint is longer and narrower compared with the corresponding joint in *P. horticola*. Terminal sensillae and three sensory areas are present on the terminal joint in both species.

The *mandibles* (Figs. 3, 4) in both species are

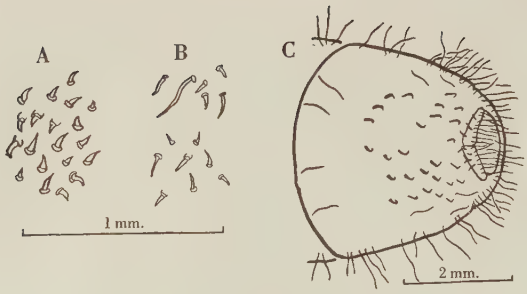

Fig. 2

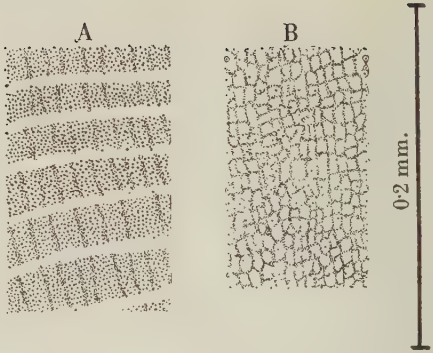

Fig. 5

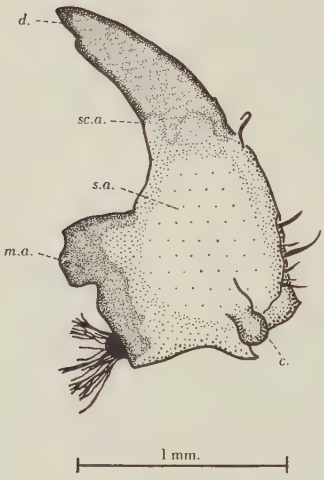

Fig. 3

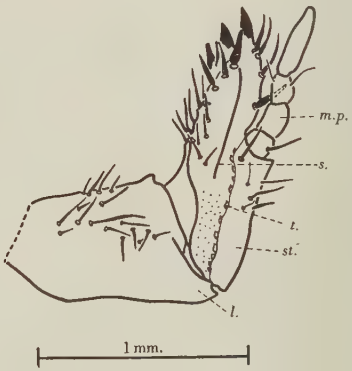

Fig. 6

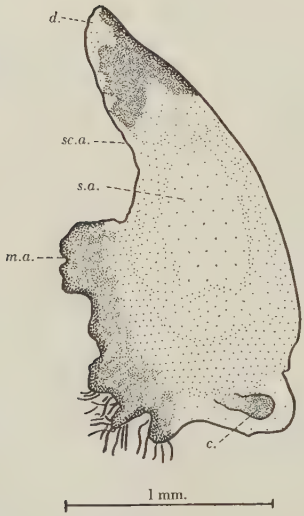

Fig. 4

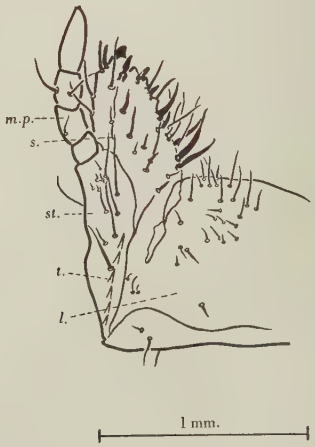

Fig. 7

heavily sclerotized. The stridulating area (*s.a.*) is on the more lightly sclerotized part on the inner surface. In *P. horticola* it consists of well-defined transverse ridges (Fig. 5 A), whereas in *H. philanthus* it consists of a much less well-defined but larger area of rows of polygons radiating from the base of the condyle (Fig. 5 B). The teeth on the stipes of the maxillae stridulate against this area.

The *maxillae* (Figs. 6, 7) in both species are strongly built. The mala which carries strongly sclerotized spines represents the fused galea and lacinia, a characteristic of the Pleurosticti. The line of fusion is much more apparent in *H. philanthus*, and in this species the tips of the lacinia and galea are free. The stipes in *P. horticola* carries the plectrum consisting of six strong teeth (*t.*) which stridulate against the mandibles as shown by Schiodte (1874). In *H. philanthus* there are about fourteen strong peg-like teeth constituting the plectrum, and the region of minute chitinized projections on the base of the stipes may also be part of the stridulating mechanism. In this species the maxillary articulation is very distinct, consisting of a postgena and an acetabular paracoila (Hayes, 1929) on the subcardo, and a parartis which fits into the paracoila.

The *labium-hypopharynx* of *P. horticola* has been described by Carpenter & Macdowell (1912). In *H. philanthus* (Fig. 8) it is an asymmetrical structure bearing distally a pair of subequal two-jointed labial palps, ventrally a fleshy ligula with two setae and dorsally a fairly heavily sclerotized area which is the hypopharynx, and against which the bases of the mandibles work during mastication. It consists of a large transverse sclerite and two lateral sclerites. The former bears a toothed projection to the right and fine setae on either side. In *P. horticola* setae occur only on the left, and according to Hansen (1893) they represent the arthropod maxillula. The hypopharynx is connected to the epipharynx by the basilar membrane.

The *labrum-epipharynx* of *P. horticola* (Fig. 10) has been well described by Rittershaus (1927). The epipharynx is the ental surface of the labrum which forms the roof of the mouth. It is asymmetrical both in *P. horticola* and *H. philanthus* (Fig. 9), the asymmetry being more marked in *P. horticola*. At each end of the clypeo-labral suture are situated more heavily chitinized areas which extend transversely towards the centre of the epipharynx and along its base. These scleromes or tormae are asymmetrical. Between the tormae are a chitinized plate and the proximal sensory area which can be distinguished by the presence of numerous fine setae. The distal and lateral margins in the case of *H. philanthus* are bordered by articulated setae, those at the distal margin being long and strongly chitinized while the lateral setae are shorter and less heavily chitinized. Proximal to the distal setae is the distal sensory area and chitinous pegs, and proximal again to these is an area surrounded by long articulated inwardly directed setae.

Of the *thoracic legs* in *P. horticola* and *H. philanthus* (Fig. 11) the first pair of legs is the smallest and the last the longest. Each leg is made up of four joints, a long coxa (*c.*), a short trochanter (*t.*), a femur (*f.*) and a tibio-tarsus (*t.t.*) which bears a terminal claw (*cl.*). Since the basal portion of the claw bears setae and has a small ridge running round it, it is maintained by Fidler (1936*a*) that the vestigial tarsus has become fused with the claw and not with the tibia, so that what is here called the tibio-tarsus may in reality be the true tibia. In *P. horticola* the claws on all three pairs of legs vary somewhat in size, but in *H. philanthus* the claw on the first leg is long and pointed, that on the second leg shorter and stouter, while that on the hind leg is very small and appears as a conical cap on the tibio-tarsus.

The 2nd and 1st instar larvae in both species closely resemble the 3rd instar larva. Notable features, however, in the 1st instar larva in both are the egg bursters. They are situated dorsolaterally on the median fold of the metathorax. Those of *P. horticola* have been described by Rittershaus (1927). Each consists of a papilla on which is situated a stout conical spine and a strong bristle (Fig. 12 A, B). In *H. philanthus* there is no raised papilla, but the conical spine is more pointed than in *P. horticola* and the bristle is longer.

The *eggs* of *H. philanthus* are very variable in shape and size, some being oval and some nearly spherical. They are semi-translucent and rather soft and in the laboratory were generally laid singly, though occasionally two or three were found together. The maximum length and width of eleven eggs were 1·62 and 1·14 mm. respectively, the averages being 1·37 and 1·02 mm. The head capsule widths of the three larval instars of *H. philanthus* and *P. horticola* are shown in

Legends to Text-figs. 2–7

Fig. 2. A and B, dorsal spines of 3rd instar larva of *Hoplia philanthus* Fuess. and *Phyllopertha horticola* L. respectively. C, anal segment (raster) of 3rd instar larva of *H. philanthus*.

Fig. 3. Mandible of 3rd instar larva of *Hoplia philanthus* Fuess. *d.* dentes; *sc.a.* scissorial area; *s.a.* stridulating area; *m.a.* molar area; *c.* condyle.

Fig. 4. Mandible of 3rd instar larva of *Phyllopertha horticola* L. (lettering as for Fig. 3).

Fig. 5. A and B, portion of stridulating area of larval mandibles of *Phyllopertha horticola* L. and *Hoplia philanthus* Fuess. respectively.

Fig. 6. Right maxilla of 3rd instar larva of *Hoplia philanthus* Fuess. *m.p.* maxillary palp; *s.* suture between galea and lacinia; *t.* tooth of plectrum; *st.* stipes; *l.* labacoria.

Fig. 7. Left maxilla of 3rd instar larva of *Phyllopertha horticola* L. (lettering as for Fig. 6).

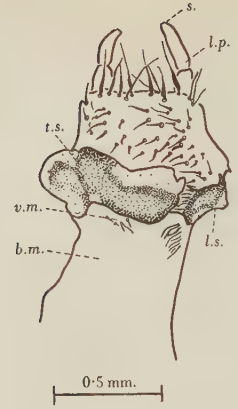

Fig. 8

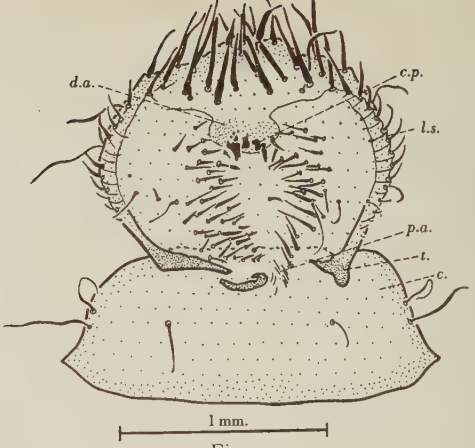

Fig. 9

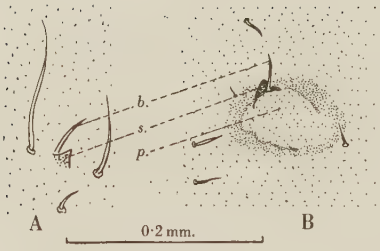

Fig. 12

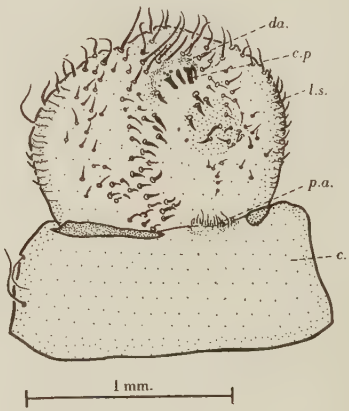

Fig. 10

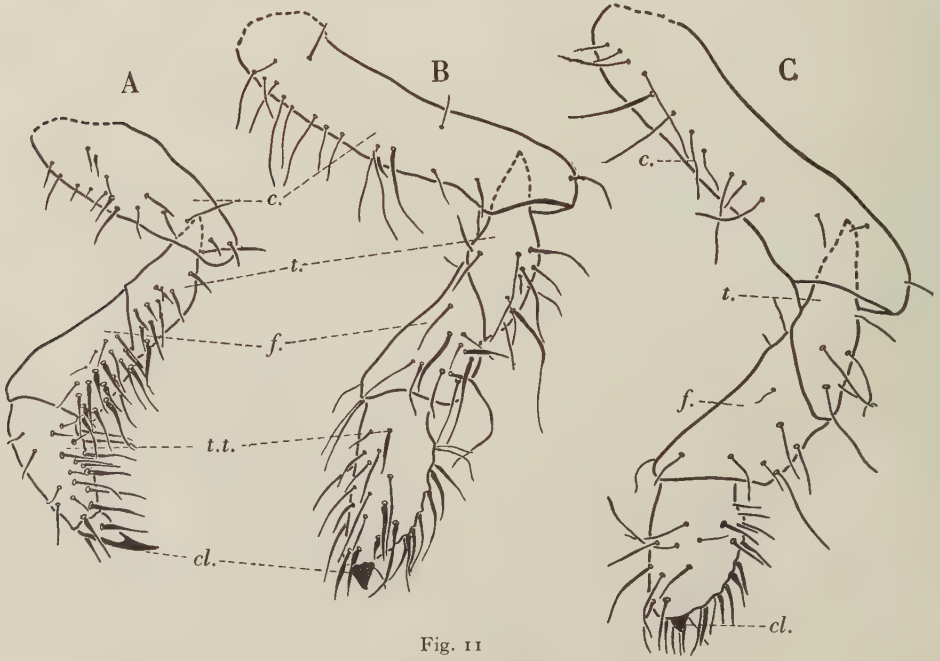

Fig. 11

Table 3. These figures fill a number of gaps in a Table of head capsule widths given by Van Emden (1941).

The *pupae* of both species are very similar, being typical white fleshy scarabaeid pupae extremely susceptible to handling. They are always to be found in an earthen pupal cell and generally within the top 3 in. of the turf. The two species are easily told apart, that of *H. philanthus* having an unmistakable humeral prominence which shows up distinctly when viewed from the side, and is absent in *P. horticola*. On the other hand, there is a large distinct tibial spur present in *P. horticola* which is absent in *H. philanthus*.

Economic importance

In the autumn of 1942 it was estimated that some 400 acres of land in north Wales were affected by the combined attacks of *P. horticola* and *H. philanthus*, the former doing by far the greatest amount of damage. The damage appears in the late summer and is worse in the autumn and winter when grass is most urgently needed for the sheep which are wintering on the lower slopes of the hills. No attempts have been made to use chemical control measures because they have in the past given only a partial control and their cost would in any case have prohibited their use in 1942 and 1943. Work on reseeding will be described in a later paper.

TABLE 3. *Widths (in mm.) of head capsules of larvae of* Hoplia philanthus *and* Phyllopertha horticola

(Number measured in brackets)

	1st instar			2nd instar			3rd instar		
	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.
H. philanthus	1·22	1·42	1·32 (32)	1·89	2·22	2·09 (43)	2·89	3·56	3·28 (50)
P. horticola	1·28	1·5	1·37 (59)	1·83	2·14	2·0 (34)	2·78	3·20	2·99 (27)

REFERENCES

CARPENTER, G. H. & MACDOWELL, M. C. (1912). *Quart. J. micr. Sci.* N.S. **57**, 373.

CURTIS, J. (1883). *Farm Insects*. Gurney and Jackson, London.

FIDLER, J. (1936*a*). Some notes on the morphology of the immature stages of some British chafer beetles. *Ann. appl. Biol.* **23**, 114.

—— (1936*b*). Some notes on the biology and economics of some British chafers. *Ann. appl. Biol.* **23**, 409.

FINNEY, D. J. (1941). Wireworm populations and their effect on crops. *Ann. appl. Biol.* **28**, 282.

FOWLER, W. W. (1888–91). *Coleoptera of the British Islands*, **4**, 48.

FOWLER, W. W. & DONISTHORPE, H. (1913). *Coleoptera of the British Islands* (Supplement), **6**,

HANSEN, H. J. (1893). Zur Morphologie der Gliedmassen und Mundtheile bei Crustaceen und Insecten. *Zool. Anz.* **16**, 193, 201.

HAYES, W. P. (1929). Morphology, taxonomy and biology of larval Scarabaeoidea. *Illinois biol. Monogr.* **12**, 87.

MINISTRY OF AGRICULTURE (1933). Report on insect pests of crops, 1928–31. *Bull.* 66.

—— (1936). Report on insect pests of crops, 1932–4. *Bull.* 99.

ORMEROD, E. A. (1893). *Report of observations on injurious insects during the year 1892*. London.

RITTERSHAUS, K. (1927). Studien zur Morphologie und Biologie von *Phyllopertha horticola* L. und *Anomala aenea* Geer. *Z. Morph. Ökol. Tiere*, **8**.

SCHIODTE, J. C. (1874). Note sur les organes de stridulations chez les larves des Coléoptères Lamellicornes. *Ann. Soc. ent. Fr.* 5me série, **4**, 39.

TAYLOR, T. H. & THOMPSON, H. W. (1928). A garden chafer attack. *Ann. appl. Biol.* **15**, 258.

VAN EMDEN, F. I. (1941). Larvae of British beetles. II. A key to the British Lamellicorn larvae. *Ent. mon. Mag.* **77**, 117.

WALTON, C. L. (1928). Rooks and agriculture in mid- and north Wales. *Welsh J. Agric.* **4**, 353.

WARBURTON, C. (1909). *J. R. agric. Soc.* **70**, 357.

—— (1911). *J. R. agric. Soc.* **72**, 383.

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Legends to Text-figs. 8–12

Fig. 8. Labium-hypopharynx of 3rd instar larva of *Hoplia philanthus* Fuess. *s.* sensillae; *l.p.* labial palp; *v.m.* vestigial maxillula; *l.s.* lateral sclerite; *t.s.* transverse sclerite; *b.m.* basilar membrane.

Fig. 9. Ental surface of labrum-epipharynx of 3rd instar larva of *Hoplia philanthus* Fuess. *d.a.* distal sensory area; *c.p.* chitinous pegs; *l.s.* lateral striae; *p.a.* proximal sensory area; *c.* clypeus; *t.* torma.

Fig. 10. Ental surface of labrum-epipharynx of 3rd instar larva of *Phyllopertha horticola* L. (lettering as for Fig. 9).

Fig. 11. A, B and C, pro-, meso- and metathoracic legs respectively of 3rd instar larva of *Hoplia philanthus* Fuess. *c.* coxa; *t.* trochanter; *f.* femur; *t.t.* tibio-tarsus; *cl.* claw.

Fig. 12. A and B, egg bursters of 1st instar larvae of *Hoplia philanthus* Fuess. and *Phyllopertha horticola* L. respectively. *b.* bristle; *s.* spine; *p.* papilla.

The biology of *Tribolium destructor* Uytt. I. Some effects of fertilization and food factors on fecundity and fertility*

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(With 12 Text-figures and 1 Diagram)

A brief review of recent papers on *Tribolium destructor* Uytt. is given, and its potential danger as a pest noted. It is shown that 85 % extraction flour leads to about double the fecundity found on 60 or 75 % extraction flour. The pre-oviposition period is shown to be affected by both larval and adult food. Unfertilized females lay eggs, but these are relatively few in number, and are all sterile. Oviposition appears to be stimulated when males associate with unfertilized females, and also when they are removed from fertilized females: the presence of five males seems to lead to greater oviposition than when only one male is present, but the use of small numbers makes these conclusions rather insecure. Although copulation occurs at frequent intervals, females will lay viable eggs for as long as 250 days after the removal of the male. Fertility remains virtually constant throughout life when males are present, and is not increased by the presence of more than one male. Fertility varies inversely with the number of eggs laid. The oviposition period is long and the average curve has a characteristic form, despite considerable daily and individual fluctuations. Oviposition may continue until death or may end some weeks before. Poor conditions tend to lengthen both the oviposition period and the length of life, the latter being greater for males than for females.

Introduction

Tribolium destructor, Uyttenboogaart (Tenebrionidae, Coleoptera) was first discovered on violet seed of unknown origin, in Erfurt, Germany, and recorded by Uyttenboogaart (1933) who described it later (1934). Its larvae were found attacking the roots of growing rye in Pomerania (Scholz, 1934); and it also occurred in stored seeds in several places in Germany (Zacher, 1935), in bird seed (containing maize from Germany) at Mälmo, Sweden (Kemner, 1936), in Argentina (Blair, quoted by Potter, 1939), in Holland (Kleine reported by Scholz, 1934), and in malt at le Puy, France (Zacher, 1935). Potter (1939) gives a summary of the literature, and describes *T. destructor* causing trouble in an English warehouse, on lantana seed from Italy, in 1938. Good (1936) gives a full taxonomic description of the species, together with an illustration of the adult. A little work was done by Reclaire (recorded by Uyttenboogaart, 1934) on its feeding habits, but otherwise its biology does not seem to have been studied.†

Since the closely related species *T. confusum* Duv. and *T. castaneum* Herbst. do considerable damage to stored products, it is possible that *T. destructor* may become a serious pest; in which case knowledge of its behaviour would be useful in devising control measures. Consequently, for the past three years, the author has been doing some voluntary work for the Pest Infestation Laboratory of the Department of Scientific and Industrial Research, on the biology of *T. destructor*; but the only experiments described here are those which concern two aspects of its breeding: (1) the effect on fecundity and fertility of flours of different extractions, and (2) the effect on fecundity and fertility of the absence of, or presence of one or more, males. Whilst this work has led to certain definite conclusions, the author regards these experiments as essentially preliminary, in that they suggest profitable fields for research, and indicate along what lines future research can best be done.

General method

In most details all experiments were done under the same conditions. The insects were kept in open glass tubes of 1 in. diameter. They could not escape, as neither adults nor larvae can climb far up smooth vertical surfaces and, as in *T. confusum*, the adults do not fly. To guard against mites the tubes were kept in trays containing paraffin, but they were stood in 'shoes' (made from the tops of screw-top bottles) to obviate wiping them every time they were removed for examination. The trays were put in a dark incubator at 25° C. ($\pm \frac{1}{2}$° C.) and 50–60 % R.H. (usually

* Part of a thesis approved by the University of London for the degree of Doctor of Philosophy.

† Since this was written, a Swedish paper has appeared (R. Mathlein, 'Investigations on pests of stored products. III. The dark-brown flour beetle, *Tribolium destructor* Uytt., a new economically important pest', *Medd. Växtskyddanst. Stockh.* 1943, nr. 41) which deals fully with *T. destructor*, its biology and economic aspects. Oviposition is studied, especially the effect of temperature upon it; the writer, however, saw this paper too late to make detailed reference to it here.

56% R.H.), which would mean that the water content of the food would be about 8–10% (Bailey, 1920).

The food was kept in the incubator, before being used, until it reached equilibrium with the temperature and humidity; and only a few tubes were removed at any one time, so that the insects should not long be subjected to different external conditions. Of the foods used the 85% (National Wholemeal), 75% (National Straight Run), and 60% (High Grade White) flours were kindly provided from a single sample of grain, by the Research Association of British Flour Millers. The other food used was sieved wholemeal, which was made from ordinary wholemeal flour, by passing it through an 80-wire mesh. These foods will be referred to as 85%, 75%, 60% and S.Wh. respectively.

The sex of individual insects was determined in the pupal stage, by the method described and illustrated by Park (1934*a*); but it was also found possible to distinguish between the sexes of the adults by the patch of golden hairs on the femur of the male, as suggested by Hinton (1942) for *T. confusum* and *T. castaneum*.

Each insect was given about 1 g. of food which was changed fortnightly to avoid contamination from faeces or scent from the adult glands. Payne (1925) showed that the adults do even more damage to flour than do their larvae, and it was, therefore, especially important to avoid conditioning, as even a slight amount markedly reduces fecundity in *T. confusum*, according to Park (1936) and Park & Woollcott (1937). So that the eggs could be found, the 85% was ground in a mechanical pestle and mortar until it would all pass through a 60-wire mesh.

The eggs laid by each female were removed daily (from the first to the last day of oviposition), to minimize loss or damage due to eating or trampling by adults. Park (1935*a*), Chapman (1928) and Chapman & Whang (1934) have shown that on fresh flour, with a density of about 1 egg/g., a pair of *T. confusum* will consume about 1 egg/day. Park (1935*a*) did not notice any difference between the sexes in egg-eating, but Stanley (1942) states that females eat eggs more avidly than do males—though he had not then published the evidence. Stanley (1938) has also shown that, except on highly nutritious food, egg-eating has a deleterious effect on oviposition, quite apart from the direct reduction in numbers which it causes. It seemed desirable, therefore, to avoid the complications to which egg consumption leads.

The eggs were found by sieving the flour through a 60-wire mesh, the adults having been first carefully removed, so that they should be disturbed as little as possible. Even so there is reason to believe that handling does somewhat reduce fecundity, as was suggested for *T. confusum* by Park (1934*a*) and Chapman & Baird (1934). The eggs were gently transferred to small pots with a paint brush, but to get a full count it was necessary also to examine the sides and bottom of the tubes, to which eggs might adhere. These eggs could not be removed intact, so they were destroyed, making it necessary to record two figures, (1) the total number of eggs laid, and (2) the number of eggs kept, which averaged over 80% of those laid. Chapman (1919) found that about 10% of the eggs laid by *T. confusum* adhered to the sides or bottom of the containers.

The eggs from each female were kept separately, and those laid during each successive 10-day period were placed in different tubes. The larvae hatching were counted and removed at intervals (never greater than 3 days). It was thus possible to calculate the percentage fertility of each female for each 10-day period of its life; but owing to the eggs lost through adhering to the tubes, fewer larvae hatched than would have been produced from the full total of eggs. It was, however, possible to calculate by proportion the number of larvae each female would have produced in any particular case. It seems probable that the sieving and handling of the eggs did not significantly affect their viability, since 100% hatching was observed many times, even for batches of as many as 40 or more eggs.

Since a small number of adults died at an abnormally early stage it was necessary to have some criterion by which to decide which should be excluded when summarizing results. Therefore, unless a female laid eggs for at least 100 days, she was regarded as unhealthy, and the results of such cases are not included in this account. Such a criterion is necessarily arbitrary, but this one seems quite sound, since those which died early did so long before 100 days (as Pearl *et al.* 1941 found for *T. confusum*), whilst those which were considered healthy lived for much longer. Apart from this, the performances of all females, however extreme, are recorded.

To economize in space no detailed tables are given, but the main points are illustrated by graphs. Full tables, and additional figures dealing with certain details, will be found in the author's Ph.D. thesis (1943) in the University of London Library.

The effect of flours of different extractions on fecundity and fertility

Using ten pairs of insects in all cases, experiments were made with insects fed throughout life on 60, 75 and 85%; and also with insects fed on 60% as larvae and on 85% as adults, and with insects fed on 85% as larvae and on 60% as adults.

Table 1 gives, for each of the foods used, the average, maximum and minimum figures for eggs laid; percentage fertility; larvae produced (estimated number); oviposition period; and length of life (female only). The average daily oviposition and larval production figures are also given. The maxima and minima have been included to give an idea of the range of variation.

TABLE 1. *Reproduction figures for pairs of* Tribolium destructor *on flours of different extractions. At 25° C. and 50–60% R.H. Ten pairs used in each experiment*

	No. of eggs				Fertility of eggs (%)			Estimated no. of larvae				Oviposition period (days)			Longevity of female (days)		
%	Av. total	Av. per day	Max.	Min.	Av.	Max.	Min.	Av. total	Av. per day	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.
85, 60	429	1·6	607	150	81	86	76	346	1·3	532	129	270	340	140	290	420	150
60	467	1·5	608	294	87	94	79	407	1·3	552	266	320	410	240	340	410	240
75	514	1·8	849	239	84	94	76	433	1·5	705	215	280	400	170	310	400	220
85	1299	5·0	1632	886	68	87	51	881	3·4	1280	677	260	350	140	290	400	160
60, 85	1104	4·4	1411	876	76	84	58	844	3·4	1100	505	250	350	160	270	370	190

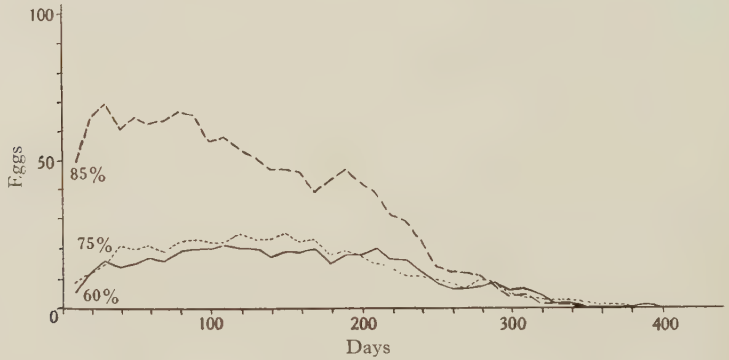

Fig. 1. Average fecundity of pairs of *T. destructor*, fed on 60, 75 and 85% flour. At 25° C. and 50–60% R.H.

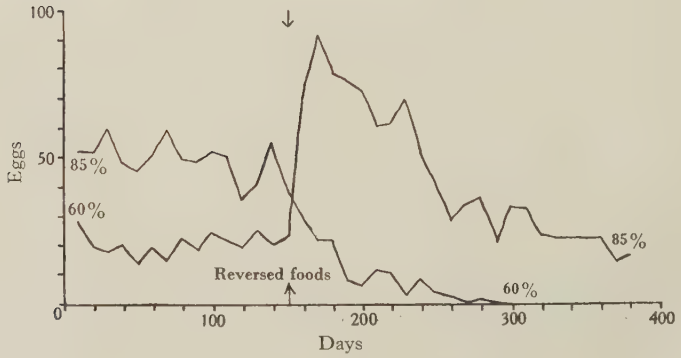

Fig. 2. Fecundity of pairs of *T. destructor* started on 85 and 60% flour; foods reversed after 150 days. At 25° C. and 50–60% R.H.

Discussion

(1) *Fecundity*. One of the great advantages of studying egg laying over the whole oviposition period (most workers on *T. confusum* have confined their observations to a short period soon after emergence) was that it was possible to show that the reduction in fecundity rate due to poor conditions is not compensated for by fecundity lasting longer. Thus insects laid at least twice as many eggs when fed on 85% (daily average per female 5·0) as they did when fed on the other extractions of flour (daily average per female 1·5 on 60%, and 1·8 on 75%). This is illustrated in Fig. 1. Indeed, the maximum number of eggs produced on either 60 or 75% is less than the minimum number of eggs produced on 85%. This difference in the relative values of 85 and 60% for fecundity is further shown by the results of reversing foods after 150 days' oviposition (Fig. 2). Although only one female was used in each case, the effect is clearly seen.

This effect of different foods on fecundity agrees with the results of Bushnell & Lund (1939) with *T. confusum*, where the addition of yeast to cornflour greatly increased oviposition, making it roughly equal to that noted by Good (1936) for *T. confusum* on wholemeal. The latter author also found a striking drop in oviposition when white flour was used instead of wholemeal.

Although oviposition was slightly greater on 75% than on 60%, for *T. destructor*, the difference is probably not significant. This is similar to the results of Chiu & McCay (1939) with *T. confusum*, who found that corn-starch has a nutritive value far below that of wholemeal, but that this nutritive value is not further reduced by purification.

There is no suggestion that feeding the larvae of *T. destructor* on 85% gives the insect any fecundity advantage if it be fed on 60% as an adult; the results (daily average per female 1·6 eggs) being indistinguishable from those where both larva and adult were fed on 60% (daily average per female 1·5 eggs). Insects fed on 60% as larvae and 85% as adults do, however, seem to lay rather fewer eggs (daily average per female 4·4) than do insects fed on 85% both as larva and adult (daily average per female 5·0), suggesting that the earlier feeding on poor food may have had deleterious effects, which the later feeding on good food does not wholly eradicate.

Fecundity figures for *T. confusum* have been given by many authors, and the effects of various factors studied, e.g. temperature, Oosthuizen (1936), Park (1934*a*, 1935*b*), Chapman (1919, 1931), Chapman & Baird (1934), Good (1933, 1936), and Dick (1937); humidity, Holdaway (1932) and Voute (1938); food, Bushnell & Lund (1939), Terao & Wakamori (1931), Chiu & McCay (1939) and Park & Burrows (1942); and conditioning, Park (1936). Figures given by different authors are often difficult to compare for various reasons; the conditions under which the experiments are done vary; fecundity is noted for different parts of the oviposition period; methods of computation vary; and it is also probable (Park, 1935*a*; Ford, 1937) that different strains have inherently different fecundities. Still less is it profitable to compare the fecundity of *T. confusum* with that of *T. destructor*, but the maximum total egg production does not seem to differ greatly in the two species, being 1632 (on 85%) for *T. destructor* and 1582 for *T. confusum* (Dick, 1937). With virgin females of *T. confusum*, Park (1933) found a suggestion of seasonal differences in rate of oviposition, but the present experiments with *T. destructor* provided no evidence for this.

The use of pairs only gives a picture which by no means necessarily remains proportionately true when larger populations are used. Thus Park (1932, 1933), MacLagan (1932) and Allee (1934) have shown that on 32 g. of flour, two pairs of *T. confusum* have a proportionately greater fecundity than either one pair or four or more pairs.

(2) *Fertility*. The term fertility here refers to the viability of the eggs laid. Little detailed work seems to have been done on the fertility of *Tribolium*, though many isolated observations have been made on *T. confusum*. Park (1936) and Park & Woollcott (1937) found that fertility was not affected by conditioning; Oosthuizen (1936) studied the effect of temperature on fertility; Holdaway (1932) studied the effect of humidity on fertility; Park & Burrows (1942) studied the effect of food on fertility; and figures for fertility have been given ranging from 65% (Park, 1935*a*) to 90% (Good, 1936). Little attention, however, seems to have been paid to the possibility of variation occurring during the oviposition period, and the method used has usually been to put eggs aside in flour, and to count the larvae sometime later, thus missing any larvae dying young.

The method used by the author covers both the difficulties mentioned, and makes it possible to give a fairly accurate picture of fertility at different times, and under different conditions.

In fact, fertility varies very little throughout life in *T. destructor*, a point of interest since Good (1936) says that sterility increases with age in *T. confusum* and *T. castaneum*, though this is not supported by short studies on *T. confusum* by Park (1934*b*) and Park & Burrows (1942), but these were concluded too early in the oviposition periods to be conclusive.

In *T. destructor* fertility may start a little low (Figs. 3, 4 and 8—a similar trend is shown by the figures of Park & Burrows (1942) for *T. confusum*), but rapidly reaches a maximum, and subsequently remains at a more or less constant level (Fig. 3). The later figures in fertility graphs inevitably become increasingly unreliable as they are based on diminishing numbers of eggs; but these graphs provide no evidence that fertility declines with age. A further

indication that fertility is still at a high level late in life is shown by the exceptionally high fertility of females fertilized after 250 days of sterile oviposition (Fig. 4). This is especially striking as the larval production is so good that the total number of larvae produced (264) is only a little less than that (343) of females which had males present with them for the whole of their life.

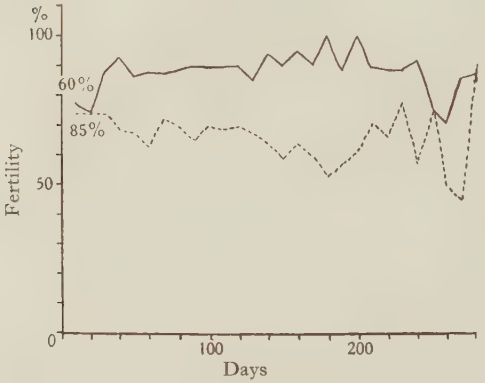

Fig. 3. Average fertility of pairs of *T. destructor* fed on 85 and 60%. At 25° C. and 50–60% R.H.

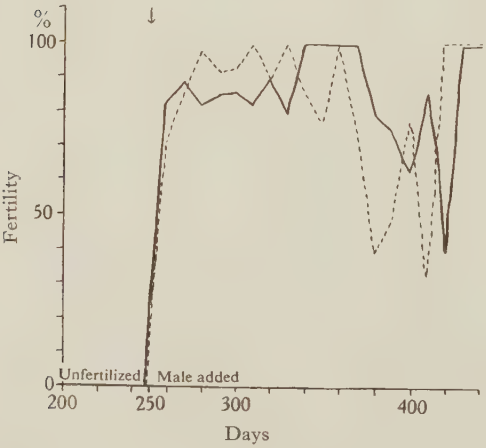

Fig. 4. Fertility of two females of *T. destructor*, with male added after 250 days of oviposition. Fed on sieved wholemeal. At 25°C. and 50–60% R.H.

Fertility is significantly higher on the less nutritive foods, as can be seen by comparing the fertility on 60% or 75% with that on 85% (Table 1 and Fig. 3). Although only one female was used in each case, the same conclusion is suggested by the fertility curves for pairs where the foods were reversed after 150 days —the lines crossing over where the change of food was made, and the fertility being more or less higher on 60% than on 85%, both before and after this point (Fig. 5).

A similar effect was noted by Park & Burrows (1942) with *T. confusum*, fertility being higher on a wood-dust-synthetic medium (on which fecundity was relatively low) than on flour (where fecundity was relatively high)—but they did not think that their figures provided adequate proof. The maximum figures obtained with *T. destructor* (representing the highest percentage fertility of *any* pair used for each 10 days) underline the inverse relation between fertility and fecundity in an interesting way (Fig. 6). They show that where relatively few eggs are produced 100% fertility is common at all times in the oviposition period; whereas where eggs are abundant

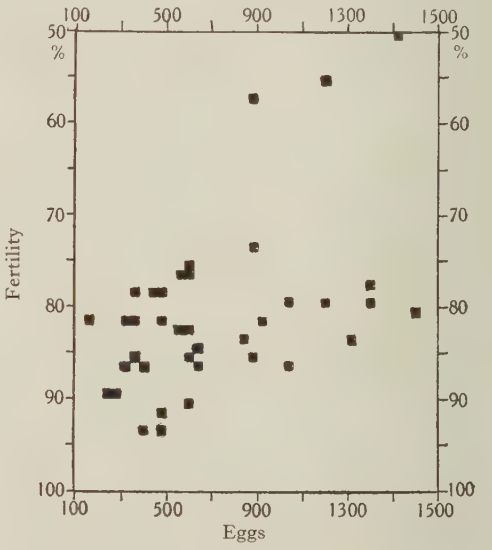

Diagram 1. Scatter diagram showing the relation between fecundity and fertility. Based on total oviposition and average fertility of about fifty pairs.

100% fertility is quite rare. The data presented as a scatter diagram, also illustrates this point (Diagram 1).

The effect of the male on fecundity and fertility

With pairs as a control, experiments were made with females with two males each; with females with five males each; with females from which the males were removed immediately after the first egg was laid; with females from which the males were removed 100 days after the first egg was laid; with solitary females; and with unfertilized females to which males were added 250 days after the first egg was laid.

These experiments were carried out as described on p. 132, except that both the insects used, and

their parents, had been fed on the same material as was used for the experiments themselves, and all the males and females in the same tube had emerged from their pupae at the same time. The food used was sieved wholemeal and, as previously stated, each *insect* was given approximately 1 g. of food, which was changed fortnightly. The method of allotting food on the basis of so much per *insect* had the advantage none are fertile. This has also been shown for *T. confusum* by Good (1936) and Dick (1937). Egg laying is much more evenly distributed over the oviposition period for unfertilized than for fertilized females, but fecundity is far lower (Fig. 7 compared with Fig. 11). The maximum number of eggs laid by unfertilized females is barely equal to the average for fertilized females, and is far below the maximum

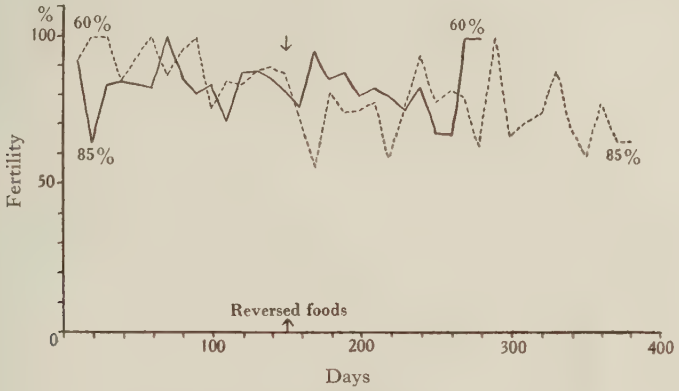

Fig. 5. Fertility of pairs of *T. destructor* started on 85 and 60%. Foods reversed after 150 days. At 25°C. and 50–60% R.H.

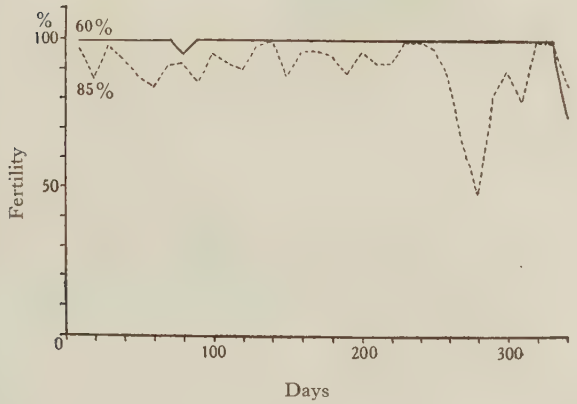

Fig. 6. Maximum fertility of pairs of *T. destructor* fed on 60 and 85%. At 25°C. and 50–60% R.H.

of keeping the density of the population constant for the various experiments, making contact between individuals as uniform as possible, even when different numbers of males were used.

Table 2 gives a summary of the results of these experiments. Although based on very few observations, the following points seem to be worth making.

(1) *Unfertilized females.* These will lay eggs, but for the latter. The average rates per female per day show this: fertilized females (pairs) 2·3 eggs, unfertilized females 1·6 eggs. Park (1933), using *T. confusum*, found that virgin females laid only one-twentieth of the number of eggs laid by fertilized females.

(2) *Fertility.* Copulation occurs at frequent intervals in *T. destructor*, as in *T. confusum* (Good, 1936;

Dick, 1937), but the presence of more than one male (whether it be two or five) does not seem to cause any increase in fertility, contrary to the view expressed by Park (1933, 1934*b*) for *T. confusum*. Living males never ceased to be fertile before the female stopped laying her eggs, which agrees with the observation of Good (1936) that males are fertile in *T. confusum* for a longer period than are the females—sometimes for over 3 years. Good (1936) also says that females of *T. confusum* will lay non-fertile eggs for long periods, even when males are present, but this did not occur in any of the author's experiments with *T. destructor*.

drawn in this section are only very tentative, as the numbers of females used were small. The data, however, may be of interest because, with insects with so long an oviposition period, detailed experiments with large numbers are difficult.

To ascertain if their presence would stimulate oviposition, males were added to two unfertilized females after 250 days of oviposition. In the previous 10-day period the two unfertilized females had laid 16 and 28 eggs respectively; during the 10-day period immediately following the addition of the male the figures were 9 and 30; but in the next two 10-day periods the figures were first 42 and 48, and then

TABLE 2. *Reproduction figures for females of* Tribolium destructor *fed on sieved wholemeal, as affected by the absence, or presence, of one or more males. At* 25° C. *and* 50–60% R.H.

	No. of females used	Eggs: Av. Total	Eggs: Av. Per day	Eggs: Max.	Eggs: Min.	% fertility: Av.	% fertility: Max.	% fertility: Min.	Estimated larval number: Av. Tot.	Estimated larval number: Av. Per day	Estimated larval number: Max.	Estimated larval number: Min.	Oviposition period: Av.	Oviposition period: Max	Oviposition period: Min.	Female longevity: Av.	Female longevity: Max.	Female longevity: Min.
Pairs	6	589	2·3	986	415	58	94	7	343	1·3	925	32	260	310	230	300	340	250
Pairs, male removed after first egg laid	4	1141	2·5	1475	747	35	46	9	324	0·7	495	100	460	520	380	500	540	420
Pairs, male removed after 100 days	5	900	2·7	1425	566	47	68	38	424	1·3	563	297	330	380	300	360	400	310
Unfertilized females, male added after 250 days	2	869	—	1002	736	86	87	85	264	—	307	221	440	—	—	470	—	—
Females, with five males	6	814	2·8	1353	265	66	87	31	539	1·8	931	81	290	380	200	320	390	200
Females, with two males	5	483	2·1	970	185	52	79	24	252	1·1	455	49	230	390	150	260	390	180
Unfertilized females	5	489	1·6	577	251	—	—	—	—	—	—	—	310	390	210	320	390	210

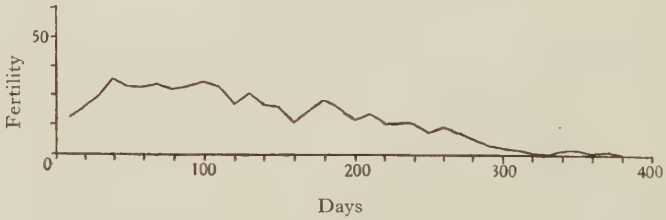

Fig. 7. Average fecundity of unfertilized females of *T. destructor*, fed on sieved wholemeal. At 25° C. and 50–60% R.H.

Removal of the male causes a slow decline in fertility (Fig. 8), but total larval production is not diminished, as the decline in fertility is offset by greater fecundity. Viable eggs were laid for about 250 days after the removal of the male, whether this was done after the first egg was laid or 100 days later. In these experiments fertility did not last as long as 250 days in most cases, the periods varying from 50 to 250 days, and two-thirds of the solitary females were still laying viable eggs 150 days after the removal of the male. These are longer periods than have been recorded for *T. confusum*, since Good (1936) speaks of solitary females laying viable eggs 150 days after separation from the male, whilst Dick (1937) found 200 days the longest, and half the females he used were infertile 150 days after the removal of the male.

(3) *Fecundity of fertilized females.* The conclusions

42 and 41. It seems clear that the addition of a male does stimulate oviposition in previously unfertilized females, but there is a lag of about 10 days before the effect becomes apparent (Figs. 9, 10).

For the two females used in this experiment, the average total egg production was quite as high as where a male had been present all the time (in spite of the low fecundity during the first 250 days, when the females were unfertilized). This effect of the male stimulating fecundity in unfertilized females was shown by Dick (1937) for *T. confusum*.

Removing the male immediately after the first egg was laid also seemed to stimulate oviposition, since the form of the curve here differs markedly from that for pairs (Fig. 11), though the effect does not seem to be produced when the male is removed after 100 days.

Removal of the male also seems to lead to greater

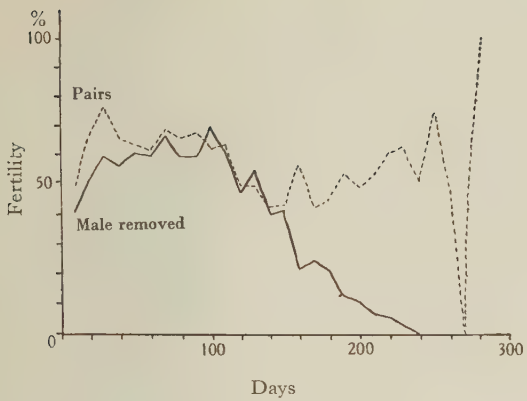

Fig. 8. Average fertility of *T. destructor*, for pairs and for females with males removed after the first egg laid. On sieved wholemeal. At 25° C. and 50–60 % R.H.

total egg production, the effect again being greater where the male is removed after the first egg is laid (Table 2).

For *T. confusum*, Dick (1937) found that the removal of the male led to a more rapid decline in fecundity than when it remained; whilst as the females ceased to lay fertile eggs their oviposition tended to resemble the level low rate of the unfertilized females. Neither of these statements seem true of *T. destructor*. The removal of the male after 100 days gave a curve indistinguishable from that where the male remained. When the male was removed immediately after the first egg was laid, the decline in fertility seemed to lead to a second peak in egg production, instead of to the usual steady decline (Fig. 11).

Oviposition is not increased by the presence of two males, but it seems to be by five (Table 2). This latter conclusion agrees with that of Park (1933, 1934*b*) who found that in *T. confusum* repeated copulation does stimulate oviposition.

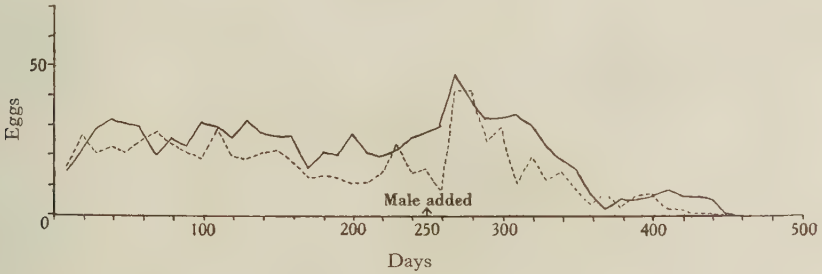

Fig. 9. Fecundity of two unfertilized females of *T. destructor*, fed on sieved wholemeal; male added after 250 days of oviposition. At 25° C. and 50–60 % R.H.

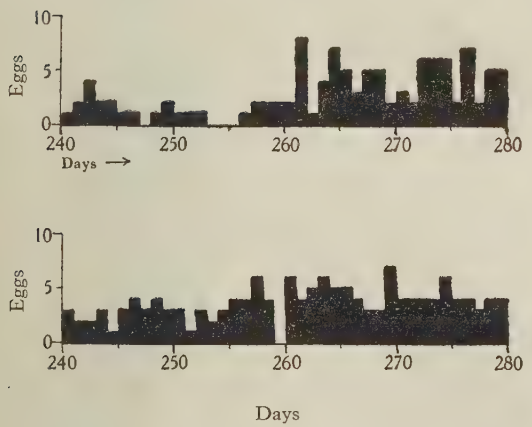

Fig. 10. Daily oviposition of two unfertilized females of *T. destructor*, fed on sieved wholemeal; male added after 250 days of oviposition. At 25° C. and 50–60 % R.H.

Pre-oviposition period

The results obtained are summarized in Table 3. The average pre-oviposition period for pairs, where both adults emerged at the same time, was 16 days. Schneider (1941) found a marked difference between the pre-oviposition period for fertilized females (2–3 days) and virgin females (never less than 12 days) in *T. confusum*, but in *T. destructor* the pre-oviposition period does not seem to be lengthened by the absence of the male (16 days), nor to be shortened by the presence of two males (19 days), or five males (16 days). The presence of a mature male does, however, seem to bring about earlier oviposition (12 days). The effect of food is clearly shown, where both male and female emerged at the same time, 60 % (27 days), 75 % (19 days) and 85 % (12 days) showing a graded series. Thus 75 % has an effect distinguishable both from that of 60 % and from that of 85 %, though the author has evidence that the effect of 75 % on the rate of development is similar to that of 60 %, and its effect on mortality

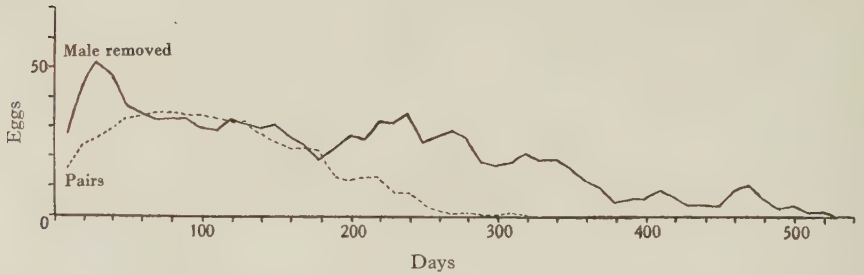

Fig. 11. Average fecundity for *T. destructor* of pairs, and of females with males removed after first egg laid. Fed on sieved wholemeal. At 25° C. and 50–60% R.H.

TABLE 3. *Pre-oviposition periods (in days) of* Tribolium destructor. *At* 25° C. *and* 50-60% R.H.

	Fed on S.Wh.	Fed on S.Wh. Solitary	Fed on S.Wh. Two ♂	Fed on S.Wh. Five ♂	Fed on S.Wh. Mature	Fed on 60%	Fed on 75%	Fed on 85%	Larvae 60% Adults 85%	Larvae 85% Adults 60%
	15	16	12	16	11	27	25	11	11	14
	11	18	13	14	14	19	18	11	13	11
	17	11	24	17	11	36	16	11	13	11
	19	17	20	16	11	29	19	10	11	10
	25	13	17	16	12	21	15	14	15	9
	14	12	30	19	13	42	25	12	13	11
	19	12	—	—	12	18	21	11	14	14
	14	12	—	—	12	28	17	12	13	16
	18	14	—	—	11	24	16	12	14	11
	16	14	—	—	11	24	16	12	12	12
	21	13	—	—	11	—	—	—	—	—
	24	16	—	—	12	—	—	—	—	—
	12	15	—	—	—	—	—	—	—	—
	13	15	—	—	—	—	—	—	—	—
	11	16	—	—	—	—	—	—	—	—
	15	19	—	—	—	—	—	—	—	—
	14	20	—	—	—	—	—	—	—	—
	14	20	—	—	—	—	—	—	—	—
	—	20	—	—	—	—	—	—	—	—
	—	19	—	—	—	—	—	—	—	—
	—	20	—	—	—	—	—	—	—	—
Av.	16	16	19	16	12	27	19	12	13	12
Max.	25	20	30	19	14	42	25	14	15	16
Min.	11	12	12	14	11	18	15	10	11	9
S.D.	4·0	2·9	7·0	1·7	1·0	7·6	3·7	1·3	1·3	2·1

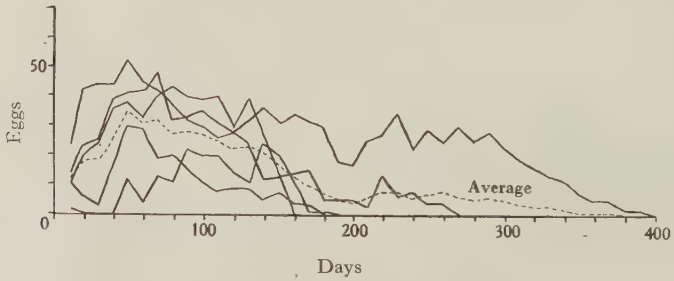

Fig. 12. Average and individual fecundity of females of *T. destructor*, with two males. Fed on sieved wholemeal. At 25° C. and 50–60% R.H.

to that of 85%, whilst the present work shows that its effect on fecundity is similar to that of 60%.

When an insect is fed on poor food as a larva, the effect can be counteracted by giving the adult good food; thus insects with larvae fed on 60% and adults fed on 85% average 13 days for their pre-oviposition period, which is similar to the time taken (12 days) by insects fed on 85%, rather than to that of insects fed on 60% (27 days). On the other hand, when an insect is fed on good flour as a larva, this will mask the effect of feeding the adult on poor food—thus insects fed on 85% as larvae and on 60% as adults averaged 12 days for their pre-oviposition period, which again is similar to the time taken (12 days) by insects fed on 85%, rather than to that by insects fed on 60% (27 days). It is, therefore, possible for larval food to have some effect on the pre-oviposition period when it is better than the adult food, but not when it is worse. This is the reverse of the effect of larval food on fecundity (p. 135).

The pre-oviposition period of *T. destructor* never seems to be as short as that of *T. confusum* (e.g. Good, 1936, less than a week), but this may be due to the unusually low temperature, 25° C., used in the present work, other work usually being done at 27° C., at least.

Oviposition period, and length of life

These may be, but frequently are not, the same, the female sometimes surviving for 50 or more days after the last egg is laid.

T. destructor, like other members of the genus, continues to oviposit over a long period. Daily fluctuations are considerable (a large number of eggs laid one day often leads to few or none being laid the next—Fig. 10), and individuals vary considerably (Fig. 12), but a fairly smooth curve is obtained by taking the average of a number of females (Fig. 12). As with *T. confusum* (Dick, 1937) the fluctuations do not seem to show any regularity.

Where fecundity is high (e.g. on 85%) the rate of oviposition usually rises fairly sharply, to reach a maximum within 20–50 days, and this high rate of oviposition may be maintained till about 100 days after oviposition starts, but usually it begins to drop before this time (Figs. 1, 11 and 12). In *T. destructor* the initial rise in fecundity seems to be a rather more gradual process (possibly due to the lower temperature used—25 against 27° C.) than Dick (1937) or Bushnell & Lund (1939) found for *T. confusum*; and there is no evidence for the two peaks of egg production found by the latter authors. Where fecundity is low (e.g. on 60 and 75%) the curve for *T. destructor* has the same form as where it is high, but it is much flatter and more uniform, and the maximum is reached more slowly—indeed, it greatly resembles the curve for unfertilized females (Figs. 1, 7).

Absence of the male seems to prolong the length of the oviposition period. Where females were solitary, all or part of the time, the average periods were 460, 440, 330 and 310 days, compared with 290, 260 and 230 days where one or more males were present throughout. Poor food may also lengthen the oviposition period, thus on 60% it was 320 days, on 75% 280 days, and on 85% 260 days. Similar conclusions are reached from the figures for length of adult life (Tables 1 and 2), but this disagrees with Good (1936) who found that *T. confusum* lived longer on whole wheat than on less nutritive flours—though this author did find that females lived longer if they did not mate.

None of the author's *T. destructor* adults lived for as long as 2 years when handled, and the longest period for unhandled adults was only just over 2 years, though Good (1936) noted some *T. confusum* living for over 4 years. Pearl *et al.* (1941), however, found that the average life of *T. confusum* as an adult was little over 6 months. As Good (1936) found for *T. confusum*, the males of *T. destructor* seem to live longer than the females (at least when both are handled), since in the present experiments the female died before the male on seven out of every eight occasions. However, Schneider (1941), and Pearl *et al.* (1941) found that where handling was reduced to a minimum the females of *T. confusum* lived longer than the males.

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REFERENCES

Although many authors have given long lists of references to papers on *Tribolium*, no complete bibliography seems to have been published. The author, therefore, feels that the bibliography which may be found in his Ph.D. thesis (1943) in the University of London Library, and which includes all the papers he has been able to find on the biology of *Tribolium* (mere references to occurrences are not included) may prove useful. Below are given only those papers to which reference has been made in the present work.

Allee, W. C. (1934). Recent studies in mass physiology. *Biol. Rev.* **9**, 1–48.

Bailey, C. H. (1920). The hygroscopic moisture of flour when exposed to atmospheres of different relative humidity. *Industr. Engng Chem.* **4**, 1102.

Bushnell, R. J. & Lund, H. O. (1939). The relation of nutritional levels to the growth of populations of *Tribolium confusum* Duval. II. Egg production in patent flour and in patent flour supplemented with yeast. *J. econ. Ent.* **32**, 640–2.

Chapman, R. N. (1919). The confused flour beetle (*Tribolium confusum* Duval). *Rep. Minn. Ent.* **17** (1918), 73–94.

—— (1928). The quantitative analysis of environmental factors. *Ecology*, **9**, 111–22.

—— (1931). *Animal Ecology, with especial reference to insects.* (464 pp.) New York and London.

Chapman, R. N. & Baird, L. (1934). The biotic constants of *Tribolium confusum* Duval. *J. exp. Zool.* **68**, 293–304.

Chapman, R. N. & Whang, W. Y. (1934). An experimental analysis of the cause of population fluctuations. *Science*, **80**, 297–8.

Chiu, F. S. & McCay, C. M. (1939). Nutritional studies of the confused flour beetle (*Tribolium confusum* Duval) and the bean weevil (*Acanthoscelides obtectus* Say). *Ann. ent. Soc. Amer.* **32**, 164–70.

Dick, J. (1937). Oviposition in certain Coleoptera. *Ann. appl. Biol.* **24**, 762–96.

Ford, J. (1937). Research on populations of *Tribolium confusum* and its bearing on ecological theory: a summary. *J. Anim. Ecol.* **6**, 1–14.

Good, N. E. (1933). Biology of the flour beetles, *Tribolium confusum* Duv. and *T. ferrugineum* Fabr. *J. agric. Res.* **46**, 321–4.

—— (1936). The flour beetles of the genus *Tribolium*. *Tech. Bull. U.S. Dep. Agric.* no. 498, pp. 1–57.

Hinton, H. E. (1942). Secondary sexual characters of *Tribolium*. *Nature, Lond.*, **149**, 500.

Holdaway, F. G. (1932). An experimental study of the growth of populations of the 'flour beetle' *Tribolium confusum* Duval, as affected by atmospheric moisture. *Ecol. Monogr.* **2**, 261–304.

Kemner, N. A. (1936). Two new indoor pests. Två nya skadejur inomhus, *Tribolium destructor*, Uyttenb. och *Sitotroga cerealella* Oliv. *Opusc. Ent.* **1**, 56–8.

MacLagan, D. S. (1932). The effect of population density upon the rate of reproduction, with especial reference to insects. *Proc. Roy. Soc.* B, **111**, 437–54.

Oosthuizen, M. J. (1936). The effect of high temperature on the confused flour beetle (*Tribolium confusum* Jacq.-Duv.). *Tech. Bull. Minn. agric. Exp. Sta.* no. 107, pp. 1–45.

Park, T. (1932). Studies in population physiology: the relation of numbers to initial population growth in the flour beetle, *Tribolium confusum* Duval. *Ecology*, **13**, 172–81.

Park, T. (1933). Studies in population physiology. II. Factors regulating initial growth of *Tribolium confusum* populations. *J. exp. Zool.* **65**, 17–42.

—— (1934*a*). Observations on the general biology of the flour beetle, *Tribolium confusum*. *Quart. Rev. Biol.* **9**, 36–54.

—— (1934*b*). Studies in population physiology. III. The effect of conditioned flour upon the productivity and population decline of *Tribolium confusum*. *J. exp. Zool.* **68**, 167–82.

—— (1935*a*). Studies in population physiology. IV. Some physiological effects of conditioned flour upon *Tribolium confusum* Duval, and its populations. *Physiol. Zoöl.* **8**, 91–115.

—— (1935*b*). Sterilization of *Tribolium* by high temperature. *Science*, **82**, 281–2.

—— (1936). Studies in population physiology. VI. The effect of differentially conditioned flour upon the fecundity and fertility of *Tribolium confusum* Duval. *J. exp. Zool.* **73**, 393–404.

Park, T. & Burrows, W. (1942). The reproduction of *Tribolium confusum* Duval in a semisynthetic woodrust medium. *Physiol. Zoöl.* **15**, 476–84.

Park, T. & Woollcott, N. (1937). Studies in population physiology. VII. The relation of environmental conditioning to the decline of *Tribolium confusum* populations. *Physiol. Zoöl.* **10**, 197–211.

Payne, N. M. (1925). Some effects of *Tribolium* on flour. *J. econ. Ent.* **18**, 737–44.

Pearl, R., Park, T. & Miner, J. R. (1941). Experimental studies in the duration of life. XVI. Life tables for the flour beetle, *Tribolium confusum* Duval. *Amer. Nat.* **75**, 289–318.

Potter, C. (1939). Occurrence of *Tribolium destructor* Uytt. in seeds in England. *Ent. mon. Mag.* **74**, 114–15.

Schneider, B. A. (1941). The nutritional requirements of *Tribolium confusum* Duval. I. The survival of adult beetles on patent flour and complete starvation diets. *Biol. Bull. Wood's Hole*, **80**, 208–27.

Scholz, R. (1934). *Tribolium* (*Stene*) *destructor* Uyttenb. *Ent. Bl. Krefeld*, **30**, 44–5.

Stanley, J. (1938). The egg-producing capacity of populations of *Tribolium confusum* Duv. as affected by intensive egg-consumption. *Canad. J. Res.* **16**, 300–6.

—— (1942). A mathematical theory of the growth of populations of the flour beetle *Tribolium confusum* Duv. V. The relation between the limiting value of egg-populations in the absence of hatching and the sex-ratio of the group of adult beetles used in a culture. *Ecology*, **23**, 24–31.

Terao, A. & Wakamori, N. (1931). Influence of the feeding of the thyroid gland and the anterior lobe of the hypophysis on the second generation of the silk worm, *Bombyx mori* L. *Proc. imp. Acad. Tokyo*, **7**, 205–7.

Uyttenboogaart, D. L. (1933). Verslag Nederlandsche Entomologische Vereeniging. *Tijdschr. Ent.* **76**, 42.

—— (1934). Revision des genus *Tribolium* (Col., Ten.). *Ent. Bl. Krefeld*, **30**, 20–31.

Voute, A. D. (1938). Bevolkingsproblem I. De toenama van een populatie van *Tribolium* en van de inheemsche bevolkung van de Tenger. *Natuurkund. Tijdschr. Ned.-Ind.* pp. 163–7.

Zacher, F. (1935). Beobachtungen über Speicherinsekten. *Anz. Schädlingsk.* **11**, 63–6.

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Inert dust insecticides

Part I. Mechanism of action

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(With 2 Text-figures)

The mode of action of inert dust insecticides on the grain weevil (*Calandra granaria* L.) has been investigated by determination of mortality-time curves and by a detailed study of the influence of dusts on the water relations of the insects. It has been found that dusts do not depend for their action on any chemical reactivity, some highly inert substances such as diamond and carborundum being very effective. A physical process is therefore indicated.

Dusts do not penetrate into the respiratory system, and although large amounts are ingested these seem to have little harmful effect.

It has been demonstrated that effective dusts increase the rate of evaporation of water from insects to the air, and that the relative killing powers of different dusts run parallel with their capacities for promoting evaporation. The results are in harmony with the view (proposed by earlier workers) that inert dusts kill insects by inducing death by desiccation.

Introduction

It has been known for many years that stored grain can be kept free from infestation by the common grain weevil (*Calandra granaria* L.) by mixing with it a small amount of certain mineral powders such as silica or quartz. The use of these non-poisonous materials, which are generally termed 'inert dust' insecticides, appears to offer many advantages, which have been reviewed in a publication of the Department of Scientific and Industrial Research (1939) and in articles elsewhere (Kitchener *et al.* 1943; Australian Council for Scientific and Industrial Research, 1941; Anon. 1940). The method is being used to some extent, for example, in Egypt and India, but for various economic and scientific reasons it has not yet been widely applied. In 1939 it was considered that further experimental work was needed before the method could be recommended for general use (D.S.I.R. 1939), since at that time knowledge of inert dusts was largely empirical, and their potentialities and limitations had been little explored.

Despite a considerable amount of research upon the use of mineral dusts as insecticides, their mode of action is not satisfactorily established. Of the divers theories proposed, that most generally favoured is the 'desiccation' theory of Zacher & Kunicke (1931) which supposes that the dusts act by promoting loss of water from the insects. This is supported by much evidence from the work of Hockenyos (1933), Germar (1936), and Chiu (1939 *a*, *b*). The latter author, by studying the oxygen consumption of the bean weevil (*Acanthoscelides obtectus* Say) established that dusts do not kill by suffocation; they did not even affect the insects' rate of respiration. In a later paper Chiu (1939*b*) extended his investigation to the grain weevil and rice weevil (*Calandra oryzae* L.), finding that inert dusts promoted water loss and that their killing power depended considerably on the relative humidity of the insects' environment. He therefore concluded that dusts kill by desiccation. The way in which dusts promote water loss remained, however, quite obscure. Zacher & Kunicke (1931) postulated a capillarity mechanism, supposing that dusts enhance evaporation by acting like blotting paper, but it seems unlikely on physico-chemical grounds that this could be the case.

In the present work, which is concerned chiefly with the grain weevil but has been extended to include several other insect pests infesting stored products, the mechanism by which dusts operate has been reinvestigated in the hope that it might thus be possible to arrive at the most suitable material for practical pest control. It was intended to make use of a physico-chemical approach to the subject unbiased, in the first place, by physiological considerations, since it seemed reasonable to presume that the action of dusts was due to some physical or chemical property the nature of which it should be possible to determine experimentally by using the weevil merely as a reagent to measure killing power. The general procedure has therefore been to conduct a series of strictly comparative tests of this kind, each designed to examine a single variable in the properties of the dusts employed. A preliminary account of the work has been given by Briscoe (1943).

Preliminary experiments

*Experimental methods**

The comparative effectiveness of dusts was determined by means of mortality-time curves for weevils placed on dusted wheat. The number of insects dead was noted at suitable intervals, usually 2 or 3 days, by separating wheat, weevils, and dust by 10-mesh and 40-mesh sieves, the sifting being carried out between two Petri dishes in order to retain the dust which was subsequently returned quantitatively to the wheat. On plotting the cumulative percentage dead against time the usual 'sigmoid' form mortality-time curves were obtained.† Such curves result from a normal statistical distribution of the day of death, and can be made approximately linear by plotting the probability function known as the 'probit' (Bliss, 1937) against time or log (time). This enables a smooth curve to be drawn through the experimental points, and the time corresponding to 50% mortality can then be found by interpolation. Although a given mortality-time curve can thus be defined by the two constants of the probability law, it is not possible to deduce a single 'toxicity constant' for each dust. Hence, all conclusions from this type of experiment have been based on *comparative* effectiveness. The *random* errors of measurement were small: although only fifty weevils were used in each test, it appears that the experimental error of points on the mortality-time curves does not exceed $\pm 5\%$. The most obvious evidence of this is the fact that curves, though they often ran very close, rarely crossed.

The insects mainly used were *Calandra granaria*. All toxicity tests were carried out at a constant temperature (23 or 26° C.) in a thermostat. Routine tests were generally made in boiling tubes (1 × 6 in.), using 50 g. of clean English wheat in equilibrium with the air; the grain was thoroughly mixed (by shaking) with a weighed amount of the dust under test. Fifty vigorous weevils were chosen at random from a large batch and placed on the dusted wheat, and the tubes then closed with muslin. The humidity under these conditions was fairly high although it was not known precisely, since it depended both on the moisture content of the wheat and on the relative humidity of the air. In experiments where a known humidity was required the tubes were replaced by 6 cm. crystallizing dishes which were placed in 'desiccators' over solutions of sodium hydroxide of known water-vapour pressure. In this way accumulation of carbon dioxide was also avoided.

A weevil was defined as 'dead' if no movement whatever could be observed after about a minute. In most cases the appearance was characteristic and only a small proportion needed careful examination. After the 'count' the dust was carefully brushed from the Petri dishes to the wheat and redistributed by shaking, the live weevils then being returned to the tube. The loss of dust was negligible except with low proportions (e.g. when less than 0·1% of the wheat by weight), and the procedure was found to be entirely satisfactory for routine comparative tests. Controls on undusted wheat were used in all experiments. It should be emphasized that dusts were compared *only within each series of tests*, since it is very difficult to maintain truly constant conditions from one series to another.

Chemical nature of effective dusts

The first step in the investigation of the action of dusts on weevils was to study the chemical nature of effective dusts. A great variety of different substances have been proposed for the purpose, ranging from strong alkalis like calcium and magnesium oxides to very inert minerals like talc and bentonite. Silica, which has been largely used as an insecticide, was first examined since, though it is usually regarded as inert, non-toxic, and practically insoluble, its solubility, though small, is generally held to be the cause of silicosis, and therefore the possibility of some special chemical action on weevils could not be ignored.

Considering the various chemical ways in which such a dust might conceivably act, it appeared that these included the formation of a toxic solution, either of the main material or of some impurity it contained, such an impurity being, in the case of silica, combined alkali, and adsorption of or chemical reaction with, some substance essential to the metabolism of the insect. A sample of silica was therefore subjected to various treatments likely to cause a substantial change in any such properties and was then used for a comparative test on weevils. The silica used for these experiments was a proprietary form of precipitated silica, marketed under the name of 'Neosyl',* and known to be a useful inert dust insecticide. The following treatments were employed:

(*a*) Repeated boiling with water.

(*b*) Repeated extraction with freshly distilled alcohol and ether.

(*c*) Extraction with freshly distilled benzene.

(*d*) Boiling with concentrated hydrochloric acid, followed by thorough washing with distilled water.

Observation of mortality-time curves showed that none of these treatments had any appreciable effect on the toxicity of the Neosyl.

As these results suggested that no kind of chemical action was involved, further experiments were made with dusts known to be very inert chemically, namely, diamond, carborundum, and aloxite. These sub-

* We are greatly indebted to Mr G. V. B. Herford, Officer-in-Charge of the D.S.I.R. Pest Infestation Laboratory, Slough, for a stock of insects and advice on their culture and handling.

† For a typical example see Briscoe (1943).

* Kindly provided by Messrs Peter Spence and Sons, Ltd.

stances, especially diamond, can hardly be conceived to exhibit chemical activity of any kind under the experimental conditions, but were extremely effective in killing weevils. In further experiments it was found that dusts differing greatly in chemical nature but of similar particle size showed roughly comparable effectiveness. It was concluded, therefore, that the action of inert dusts on weevils is *not* in any sense a chemical process. The *physical* properties which are necessary in an effective dust will be considered later in Part II.

Site of action of dusts

It was noticeable that, in general, dusts had to adhere to the insects in order to be effective; for example, some forms of silica were quite without effect because they were too coarse to adhere well.

Particle size plays a most important role in adhesion and the finer the particles the greater the covering power, but it will be shown in Part II that the effectiveness of a powder of a given material is not determined solely by its fineness. Further, in comparing different dusts, the *weight* of dust adhering seems to be relatively unimportant and, in fact, though many very effective powders coat the insects thickly, some of the most effective ones leave only a very thin film not visible to the naked eye. In tests carried out on wheat the effectiveness of a given dosage of different dusts depends much on the adhesion of the dust not only to the insect but also to the berries. Thus if the dust adheres well to the grain it may not be capable of covering the weevils as well.

One conceivable mode of action of dusts would be by penetration into the tracheae via the spiracles. To investigate this possibility a number of weevils were kept in contact with various dusts for several days and then killed and dissected. The respiratory system was carefully examined but in no case was any dust detected in the spiracles or tracheae. This observation is consonant with the evidence afforded by the work of Chiu that the respiratory system is not involved. In the course of these dissections, however, an important observation was made: in weevils which had been living on wheat heavily dusted with jeweller's rouge the whole of the *digestive tract* was bright red, showing that large amounts of rouge had been eaten along with the food. This observation was subsequently confirmed with carborundum, emery, diamond dust, tungsten powder, barytes, aloxite, glass, charcoal, and mica. It therefore seems certain that all kinds of dusts are eaten indiscriminately.

Neosyl could never be detected in the digestive system since its particles are indistinguishable from food or tissue, but a method was devised which proved that Neosyl behaves quite normally. Some of the powder was ground with sufficient of the brilliant inert blue pigment, Monastral Fast Blue, to colour it. Weevils living on wheat dusted with this mixture developed a blue colour in the digestive system, and since the particles of both powders are extremely small and hence inseparable, there can be no doubt that Neosyl is eaten. The difficulty of seeing small particles of silica owing to its low refractive index and transparency probably explains why Germar (1936) did not find any trace of 'Naaki' (a powdered quartz sand) after careful microscopic examination of the complete digestive system of dusted weevils. The use of carborundum powder eliminates this difficulty, as the refractive index is high and the particles are very characteristic in appearance. However, the extent to which these ingested dusts harm the insects is not easy to decide. Experiments in which weevils were fed with a kind of 'macaroni' made from wheat flour with and without the inclusion of carborundum proved inconclusive, because the greater hardness of the food containing the dust made it less palatable to the insect.

Water loss from insects

As mentioned above, previous workers have concluded that mineral dusts exert their insecticidal action by promoting water loss from insects. This view is entirely confirmed by the present work.

It is well known that water relationships are a vitally important factor in insect physiology. Existing information has been well summarized by Buxton (1932), and more recently by Johnson (1942), who concluded from published data that the water loss from insects is proportional to the saturation deficiency, whereas he found an exponential relationship between longevity and saturation. It is noticeable, however, that the available experimental data on the loss of weight of insects at different humidities are very fragmentary and further careful work is needed.

Mellanby (1934) found that for insects which can control their spiracles the water loss is greatly increased by adding 5% of carbon dioxide to the atmosphere since this acts as a respiratory stimulant. The work of Buxton (1932) and Mellanby (1932) on the loss of water from mealworms will be reviewed in a later section dealing with the effect of dusts on mealworms. According to Robinson (1928) the water content of insects of different species is directly proportional to that of the food on which they live, and in the case of the grain weevil he found that about half of its water exists in a free state while the other half is 'bound' to colloids. (For a discussion of 'free' and 'bound' water see Gortner (1937).) Our experiments show that when the rate of desiccation of weevils in dry air is followed by weighing and the results are plotted as in Fig. 1, 90% of the water content of the insect is given off at a constant rate. This is particularly clear in the case of insects killed previous to desiccation, and this evidence suggests that the proportion of their content water

which is 'bound' in a manner distinguishable from 'free water' cannot exceed about 10%.

For insects living on dry food the conservation of water is of the utmost importance, and any marked increase in the rate of water loss must lead to death.

Experimental

There are several reasons why the weight loss of starving insects can be considered to be a sufficiently good measure of water loss. Mellanby (1932) showed that in the case of starving mealworms the weight lost as carbon dioxide in respiration was almost exactly equal to the weight gained by the metabolic oxidation of food reserves to carbon dioxide and water, and that therefore the net weight change of the insect represented its water loss. Secondly, the refers to the loss of weight of insects in still dry air, the insects being kept in a dish covered with wire gauze and placed in a desiccator over solid calcium chloride and sodium hydroxide. There is an interesting feature in curve *A*. For the first 70 hr. the rate of loss of water was fairly constant, and it then increased rather sharply to remain constant at a higher value until only 10% of the total water content remained. The existence of this break in the curve was confirmed in a number of repetitions of the measurements and is apparently significant though it is not always as striking as in the example shown. It coincides roughly with the time when about half the insects are dead and the increase observed agrees with the observation that dead insects lose water faster than live ones (vide infra).

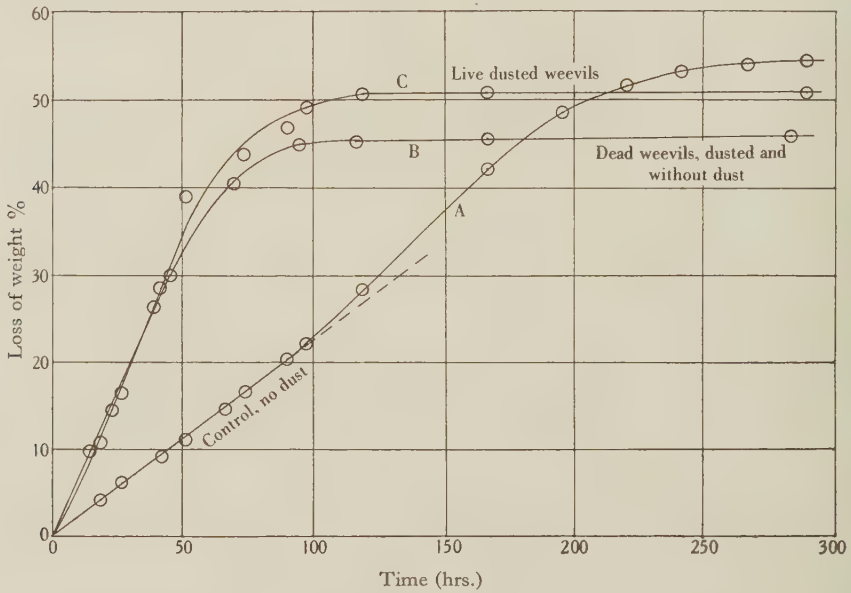

Fig. 1. The effect of dusts on the loss of weight of *Calandra granaria* in still, dry air at 23° C.

smallness of the difference between the total weight losses for live and dead weevils during desiccation (Fig. 1) shows that respiration can play but a small role in the change of weight of starving insects. Thirdly, as already mentioned, the respiration of insects remains unchanged on dusting, and thus the difference in rate of loss of weight between dusted and undusted insects must represent a corresponding difference in loss of water.

The loss of weight of a known weight and number of weevils in a stream of air of known humidity was studied at four different relative humidities. The rate of loss of weight at a given humidity was constant over the period of the experiments (24 hr.) and Fig. 2 shows the effect of humidity on this rate. Fig. 1

The effect of dusts on the loss of weight of the weevil

To ascertain the influence of dusts on the rate of evaporation of water, weighing experiments were also carried out at the same time with insects dusted with Neosyl, and the results are included in Figs. 1 and 2. It will be seen that dusting increased the rate of water loss at all humidities from the moment the insects were dusted, and that the effect of humidity on the rate of loss of weight of dusted insects was essentially the same as that for the undusted insects (Fig. 2).

In Fig. 1 the effect of a powder on the loss of weight over a longer interval is shown, and the results are again similar to those of the undusted insects

except that the rate has been much increased. It must especially be noted that effective *dusts do not themselves absorb water* from the insects; indeed, many of them, like diamond or carborundum, are quite incapable of doing so. They function merely by promoting, in a way yet to be ascertained, the normal loss of water vapour by the insect to the surrounding air. Effective dusts therefore multiply the rate of water loss at all humidities by a roughly constant factor. It might be argued that since, as will be shown later, dead insects lose water at a much faster rate than live ones, the increased water loss on dusting was not produced by the dusts but was a secondary consequence of the accelerated death of the insects. This, however, cannot be the case, since the high and steady weight loss occurs immediately on dusting.

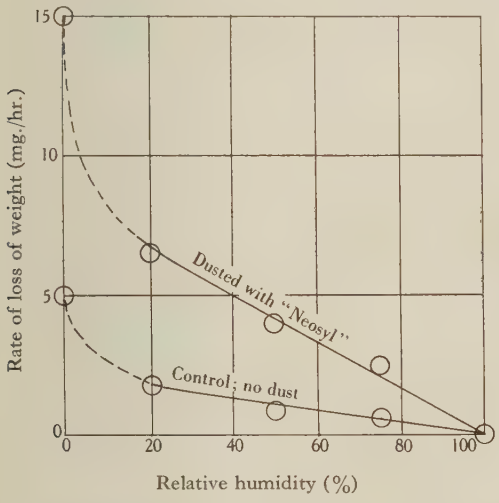

Fig. 2. The effect of Neosyl on the rate of loss of weight of *Calandra granaria* in air streams at different relative humidities.

Loss of weight of dead insects

Germar (1936) stated that the action of dusts continued after the insects' death, since he found that they also accelerate the water loss from beetles previously killed by ether. We were quite unable to reproduce these results, and in our experiments dusted and undusted dead insects lost water at the same rate, which was also identical with that observed in the case of living insects treated with the most potent dusts (see Fig. 1). Germar's results are probably due to his use of ether to kill his insects. Weevils are remarkably resistant to ether vapour, and very heavy exposures are necessary to kill them with it. On the other hand, a small dose anaesthetizes them, often for periods of several days, and the water loss from these anaesthetized insects is less than tha from dead insects. Hence Germar's 'dead' insects showed a dusting effect. The fact that the rate of loss of weight of dead weevils cannot be increased by dust has been confirmed for insects killed by the most diverse agents, including freezing to $-80°$C. and many different fumigants, as well as for weevils of different ages and insects starved or normally fed prior to killing. *In the case of the grain weevil* it seems that insects treated with effective inert dusts lose water at approximately the same rate as dead insects, but the total percentage of weight lost in infinite time for undusted insects is approximately 55%, for live dusted insects approximately 50% and for dead dusted and undusted insects approximately 47%. These differences no doubt represent the effect of respiratory losses together with a certain amount of metabolic water formed by the living insect when using its fat reserves. The fact that the total loss of weight of dusted insects is between that of the dead and the undusted insects is undoubtedly due to the earlier death of dusted insects and their consequently reduced respiratory losses. As already pointed out, these data clearly demonstrate that weight loss of insects with or without dusts can be taken as a criterion for water loss.

TABLE 1. *Relation between desiccation and mortality* (temp.* 23°C.)

Dust	% weight lost after 48 hr.	% mortality of starving weevils after 48 hr.
Nil (control)	12	0
Sulphur	11	0
Dolomite (ground under benzene)	22	20
Zincblende (ground dry)	23	25
Zincblende (ground under benzene)	31	50
Neosyl	38	80
Carborundum	40	90

* Mortality figures for the simple 'effectiveness test' (described more fully in Part II) in which weevils are placed in contact with excess dust in glass dishes which are then placed in a desiccator over calcium chloride or sodium hydroxide.

Relation between loss of weight and longevity of dusted insects

Having established that effective inert dusts accelerate the water loss from weevils and that the process can be conveniently followed quantitatively by weighing, it was next necessary to decide whether the killing power of different dusts is precisely parallel with their capability to promote water loss. That this is true in the case of starving weevils kept in contact with excess dust in dry air is shown by the data in Table 1. Weighing and toxicity measurements in triplicate were made with batches of 100 weevils and five different dusts. The results clearly prove

that the greater the water loss caused by a dust, the sooner the insects are killed. Some further data illustrating this point in the case of the mealworm are given in Part III. In addition, other experiments at different humidities were in complete accord with a direct relationship between desiccation and killing power. (Compare the toxicity data in Table 2 with the loss of water figures in Fig. 2.)

We conclude that when grain weevils are kept without food in an atmosphere of relative humidity less than about 80% they eventually die of desiccation, and that a given dust accelerates death simply by increasing the rate of drying of the insects by a constant factor characteristic of their particular 'effectiveness'.

up, the digestive tract was empty except for a little dust (e.g. lodged in the proventriculus and mid-gut), the tissues were emaciated, and the insects had every appearance of starvation as well as desiccation. This is all in harmony with the view that the role of dusts is to disturb the insect's water balance, and suggests that this disturbance also leads the insect to cease feeding.

It does not *necessarily* follow from these experiments that the action of dusts on the insects is a *direct* promotion of water loss. This is the simplest hypothesis, but it is possible to argue that water loss might be a secondary symptom arising from some other unfavourable effect of dust on the organism—such as, for example, some form of irritation. The

TABLE 2. *Variation of toxicity of carborundum (grade 800 P.A.H.) against starving insects* with relative humidity*

Relative humidity	Dust	% dead† after (days) 1	2	3	5	7	11
0%	Carborundum	2	78	100	—	—	—
(over solid NaOH)	Control	0	0	10	67	98	—
25%	Carborundum	—	25	95	—	—	—
(over 38% NaOH)	Control	—	0	0	10	45	—
45%	Carborundum	—	20	83	100	—	—
(over 32% NaOH)	Control	—	0	0	7	24	85
70%	Carborundum	—	3	62	98	—	—
(over 25% NaOH)	Control	—	0	0	10	25	70
85%	Carborundum	—	2	22	64	86	92
(over 15% NaOH)	Control	—	0	0	0	3	12

* Twenty insects were placed in a glass dish of 5 cm. diam. which was placed in a desiccator over sodium hydroxide of the requisite strength (temp. 23°C.).

† Mean values of two parallel series of experiments.

TABLE 3. *Toxicity of carborundum at 100% relative humidity against starving weevils**

	% dead after (days) 10	15	19	24	28
	Carborundum				
In triplicate	16	62	82	100	—
	7	39	75	96	98
	19	49	84	97	98
Mean value	14	50	80	98	99
	Control				
In triplicate	8	34	60	82	92
	15	38	71	70	92
	15	31	55	83	94
Mean value	13	34	62	85	93

* Fifty insects in glass dishes in desiccators over distilled water.

The physico-chemical evidence is also supported by simple physiological observations. When weevils which had been killed on dusted wheat were dissected, it was found that their tissues were desiccated. If the humidity had been low, leading to a rapid rate of kill, there was often much unused fat, and the digestive tract still contained food (and dust), whereas if the humidity in the experiment had been high, so that the insects lived longer, fat reserves were used

remarkable constancy of the rate of water loss (as well as other evidence on the nature and specificity of effective particles) seem, however, to suggest strongly a *physical* as opposed to a biological mechanism.

The effect of dusts at very high humidities

Germar found that at 100% relative humidity starving weevils dusted with Naaki survived longer than control insects without dust. Although we observed the same result in a preliminary experiment, more careful work with larger numbers of insects showed that this is not in fact the case and that dusts have very little, if any, effect in completely saturated air. This point is illustrated in Table 3. The small difference between normal and dusted insects is scarcely significant and might well be due to a slight irritant action. It was noticeable that the random error of these measurements of death rate at high humidities was very large (possibly through disease) and this probably accounted for the freak results mentioned above. Unfortunately, it is impossible to carry out an experiment with insects on wheat in saturated air since grain absorbs water and the insects can never be in an environment of 100% relative

humidity until the grain has gone completely mouldy. For experimental reasons it is also impossible to carry out weighing experiments at extremely high humidities since under those conditions water tends to condense in the pores of the insect. However, carborundum and other highly effective dusts still have a very pronounced action at relative humidities as high as 95 %. Thus, at this humidity, 50 % of starving insects were killed by carborundum in 7 days, whereas 50 % of the control were not dead until 22 days had elapsed.

The conclusion to be drawn from these experiments is that the action of dusts on grain weevils is confined almost entirely to increasing the rate of evaporation of water from them, and that any other harmful effects, such as might arise from ingestion of dust, are negligible in comparison.

The question of the mechanism by which dusts enhance evaporation from insects will be discussed in Part II, after consideration of the conditions which are required in a dust for it to be an effective insecticide.

REFERENCES

Anon. (1940). The storage of foodstuffs in the Colonial Empire. *Bull. imp. Inst., Lond.*, **28**, 163.

Australia, Council for Scientific and Industrial Research (1941). *15th Ann. Rep.* (1940–1), p. 19.

Bliss, C. I. (1937). Calculation of the time-mortality curve. *Ann. appl. Biol.* **24**, 815.

Briscoe, H. V. A. (1943). Some new properties of inorganic dusts. *J.R. Soc. Arts*, **91**, 593.

Buxton, P. A. (1932). Terrestrial insects and the humidity of the environment. *Biol. Rev.* **7**, 275.

Chiu, S. F. (1939*a*). Toxicity studies of the so-called 'inert' materials with the bean weevil, *Acanthoscelides obtectus*. *J. econ. Ent.* **32**, 240.

—— (1939*b*). Toxicity studies of so-called 'inert' materials with the rice weevil and granary weevil. *J. econ. Ent.* **32**, 810.

Department of Scientific and Industrial Research (1939). *Common pests of grain and other stored produce.* H.M. Stationery Office.

Germar, B. (1936). Versuche zur Bekämpfung des Kornkäfers mit Staubmitteln. *Z. angew. Ent.* **22**, 603.

Gortner, R. A. (1937). *Select Topics in Colloid Chemistry.*

Hockenyos, G. L. (1933). Effect of dusts on the oriental roach. *J. econ. Ent.* **26**, 792.

Johnson, C. G. (1942). Insect survival in relation to rate of water loss. *Biol. Rev.* **17**, 151.

Kitchener, J. A., Alexander, P. & Briscoe, H. V. A. (1943). A simple method of protecting cereals and other stored foodstuffs against insect pests. *J. Soc. chem. Ind., Lond.*, **62**, 32.

Mellanby, K. (1932). The effect of atmospheric humidity on the metabolism of the fasting mealworm (*Tenebrio molitor*). *Proc. roy. Soc.* B, **111**, 376.

—— (1934). The site of loss of water from insects. *Proc. roy. Soc.* B, **116**, 139.

Robinson, W. (1928). Relation of hydrophilic colloids to winter hardiness of insects. *Colloid Symp. Monogr.* **5**, 199.

Zacher, F. & Kunicke, G. (1931). Investigations on the insecticidal action of oxides and carbonates. *Arb. biol. Abt. Anst.—Reichsanst., Berl.*, **18**, 201.

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Inert dust insecticides

Part II. The nature of effective dusts

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The conditions required in an inert dust for it to become an effective insecticide against grain weevils have been investigated. *Particle size* and *intrinsic hardness* are important, but not the only, factors. In the case of carborundum, particles larger than 15 μ are without action, probably because they do not adhere to insects, and effectiveness increases as the size is reduced from 10 μ to about 2 μ where a maximum effectiveness is reached.

The effectiveness of a large number of different substances has been tested by a simple method which eliminates complications arising from particle size differences, and the results show a rough correlation of effectiveness with hardness. In general, materials softer than calcite are ineffective, and effectiveness increases with hardness, but the method of preparation of dusts is also important. Some dry-ground powders are inferior to those wet-ground, a phenomenon which appears to be due to some kind of surface change—possibly the formation of a Beilby polish layer. The effectiveness of some dusts can also be considerably altered by superficial chemical treatment.

A theory is proposed, based on an experiment with an artificial membrane system, to explain the mechanism by which dusts promote evaporation of water from insects. It is suggested that clean crystalline surfaces of effective dusts can absorb, or in some way penetrate, the water-resistant epicuticle.

Introduction

The work described in Part I was carried out with two inert dusts—Neosyl and carborundum—which were effective in killing weevils. The experiments described demonstrate conclusively that the chemical properties of the different powders play no part in this process. Therefore, since certain dusts are highly effective and others less so, it seems to follow that there must be some physical or mechanical property responsible for killing weevils. Previous investigations offer no clue whatever to its nature.

Particle size

Chiu (1939) investigated silica powders of different particle size ranges and concluded that the finer the material the greater was its toxicity. Preliminary experiments carried out as previously described on wheat, using roughly fractionated samples of carborundum and quartz, confirmed this and indeed showed that particle size was a very important factor. The chief conclusions, based on many experiments using samples fractionated by sedimentation and also powders ground to different degrees of fineness by changing the time or method of grinding, were (*a*) that particles over 15 μ are too large to act at all; (*b*) that reduction in particle size from 10 μ to 1 μ progressively increases the effectiveness; (*c*) that there may be a maximum of effectiveness at about 1–2 μ, though it can at the most only be a flat maximum (cf. Parkin, 1944). This point could not be clearly settled, partly because practical difficulties preclude the preparation of uniform samples of 1 μ or less, and partly because, as will be seen later, fractions obtained by sedimentation in water may suffer superficial changes which alter their effectiveness on weevils to an extent which varies with the time of contact with water. When dusts differing in particle size range are used in comparative experiments on wheat, secondary effects, such as relative adhesion to wheat and weevils and differences in covering power and aggregation, so greatly influence the resulting mortality that no conclusion can be drawn as to the relative intrinsic insecticidal merit of the substances employed. Adhesion, the most important of these factors, depends very largely on particle size: above 15 μ there is no adhesion; at about 5 μ the particles stick well both to wheat and weevils; at about 2 μ or less mutual adhesion of particle to particle causes much aggregation of the dust. When powders of different ranges of particle size are compared, the results are complex and cannot be related to the proportions of the several fractions in each. Another complicating factor, especially at low concentration of dusts, is preferential adhesion to either insect or grain.

For all these reasons it was obviously necessary to eliminate particle size as a variable in the comparison of different materials.

The obvious way of achieving this end is to use carefully fractionated powders having a narrow particle size range. Though this was done in certain cases, the preparation of such dusts is much too laborious for general use and, as mentioned above,

it may also be misleading because surface changes occur by reason of the prolonged immersion in water involved in fractionation.

An alternative device which suggested itself was the use of a dust containing all ranges of particle size in such great excess as to ensure that the most effective particles, which sometimes constitute only a small proportion of the material, are available in ample quantity to produce their maximum effect, the rest of the dust having little or no effect on the result of the experiment.

This second method was developed into a special technique, which may conveniently be referred to as the 'dish test', in which twenty or thirty insects are placed in a glass dish of 5 cm. diam. with plenty of the dust under test (0·5–1 g. depending on its density), but no wheat. The dish is covered with muslin and kept at 23° C. ± 0·5° in a desiccator over calcium chloride and sodium hydroxide so as to accelerate the test and to keep the atmospheric conditions constant at 0% relative humidity and 0% of this method of testing, it was applied to several carborundum dusts of different particle size distribution which had been found to differ greatly in their effect upon weevils living on wheat. In the dish test these all gave substantially identical results (Table 1), so long as there were some particles present with a diameter less than 10 μ: thus proving that the effects of variation in particle size were substantially eliminated.

An important aspect of the dish test is that starving insects do not eat dust under these conditions; it is thus possible to study exclusively the 'toxicity' which a material exerts by virtue of its adhesion to the insects' exterior, independent of any ordinary poisoning properties. Thus, for example, witherite ($BaCO_3$), arsenious oxide, and oxalic acid, three substances highly poisonous to weevils when used on wheat, have almost no killing effect (less than 10% dead after 48 hr.) in the dish test. Excepting substances capable of acting as ordinary poisons, it may be said that any powder effective on wheat is effective

Table 1

Substance	Sample no.	Particle size	% dead, 'dish test' 1 day	2 days	3 days	% dead on wheat after 4 days under standard conditions
Carborundum	800 P.A.H.	Chiefly 1–4 μ	20	90	100	63
,,	FGSB	Chiefly 1–10 μ	5	85	100	29
,,	S. 11	Fractionated 5–20 μ	15	85	100	9
,,	S. 10	Fractionated 2–4 μ	15	85	100	97
,,	S. 13	Fractionated: all over 15 μ	0	0	20	0
Flint	S. 42	Fractionated 1–10 μ	10	95	100	13
,,	S. 43	Fractionated: less than 1 μ	15	75	100	42
No dust	—	Control	0	0	5	0

carbon dioxide. The number of weevils dead are counted daily. With each series two controls were run, one with no dust and the other with a standard carborundum dust 'Grade 800 P.A.H.' (that employed in the experiments in Part I) having the following particle size distribution by weight:

Less than 1 μ	0·1%
1–3 μ	10
3–5 μ	29
5–7 μ	32
7–9 μ	21
9 μ and over	8

By means of these two controls it was possible to make approximate comparisons between results from different series of experiments, even when batches of insects differing in age, etc., had been used. Whenever a precise comparison of dusts was required it was made directly in the single experiment. By running many duplicates it was shown that a difference of about 10% in the number dead at a mortality of the order of 50% was significant in a direct comparison. As an experimental check on the reliability in the dish test, but that powders effective in the dish test may be almost ineffective on wheat if they are too coarse. Any material effective in the dish test, however, will be effective on wheat so long as it is fine enough.

Hydrophilic and hydrophobic dusts

As already mentioned, Zacher & Kunicke (1931 postulated that dusts promote loss of water by a capillary mechanism: presumably a sort of blotting-paper effect was envisaged. To examine this point, Neosyl and standard carborundum were coated with a stearic acid film from benzene solution. The effectiveness of these powders was not impaired in the least when they were coated with only a thin film of the fatty acid about sufficient to form a layer 2–3 mol. thick, though this sufficed to make them lyophobic to such an extent that they were unwettable and would float on the surface of water instead of sinking. They only became less effective in killing weevils when they had a heavy film of stearic acid. Sulphur—a naturally hydrophobic powder—was quite ineffective even when rendered

hydrophilic by coating with gelatine. Although most of the active materials are hydrophilic several, including lampblack and graphite, are hydrophobic, and it therefore appears that the wettability of the several powders cannot be correlated with their effectiveness against weevils. Consequently, any theory based on capillary rise is excluded, even without appeal to the objection on physiological grounds that there appears to be no part of the insects' exterior where dusts could come in contact with *liquid* water.

The influence of intrinsic hardness

In the course of the work some 400 different powders were examined, and from the results it was apparent that there was a clear correlation between intrinsic hardness and effectiveness. Thus, in general, hard materials like diamond, carborundum, and quartz were very effective, soft substances like talc, china clay and gypsum had little effect, while bodies of

Table 2

Hardness no.	Mineral	Method of preparation of dust	% dead 40 hr.	% dead 65 hr.
10	Diamond	Wet ground	50	100
9	Sapphire	,,	40	95
8	Spinel	,,	30	90
7	Quartz	Micronized	25	85
6	Felspar	,,	35	100
5	Apatite	Wet ground	10	50
4	Fluorspar	,,	5	45
3	Calcite	,,	5	35
2	Gypsum	,,	0	5
1	Talc	Commercial powder	5	5
	Control; no dust		5	5

intermediate hardness like fluorite and glass were moderately good for killing weevils. The same general relationship held for powdered metallic and non-metallic elements. When, however, scratch hardness on Moh's scale is plotted against effectiveness the results are irregular and not consistent with any simple relationship between these two quantities. The general trend of the results showed that materials of hardness below about 3 (calcite) were ineffective: with increasing hardness from 3 to 6 (felspar) effectiveness increased rapidly, and with further increase in hardness from 6 to 10 (diamond) there was a further, though smaller, increase in effectiveness. Table 2 shows the effectiveness of samples of the ten substances used as standards in Moh's scale of hardness.

That hardness is not the only criterion is also shown by the facts that (*a*) a few soft materials like galena, magnesite and zincblende in the hardness range 2·5–4 are abnormally effective, and (*b*) the method of grinding and also artificial alteration of the surface of the particle may markedly affect the killing power of a material. Moreover, though dusts act on the hard outer shell of the weevil, their effectiveness is not sharply differentiated by their being harder or softer than the shell, as it evidently should be if the effect depended solely on scratching or abrasion. It was found that a calcite crystal (hardness 3·0) could be grated away on a weevil shell without scratching the shell, whereas a fluorite crystal (hardness 4·0) cut the shell without itself becoming abraded. The hardness of the shell is therefore about 3·5. Yet, as shown above, the effectiveness of dusts begins to be perceptible about hardness 3·0 and increases progressively up to hardness 10.

It seems likely, therefore, that the property of dusts really responsible for killing weevils is not hardness itself but rather some other property closely, though not necessarily precisely, correlated with hardness. Moreover, there are a number of very effective substances of indeterminate hardness, such as activated charcoal, whose action may be due to their special surface character. Of a number of carbon blacks examined some were very effective whereas others were quite useless, and it was found to be impossible to correlate the killing power with the fundamental particle size (10–120mμ), the tinting strength, the *p*H, or the 'blackness' of these dusts.

The influence of conditions of grinding

Small samples of the materials under investigation were prepared by prolonged grinding (1–3 hr.) in a mechanical agate pestle and mortar; larger samples were ground in a laboratory porcelain jar mill. It was soon found that the effectiveness of a given dust often depended greatly on whether it was ground dry or under a liquid, the wet-ground powders being in many cases decidedly more effective than the dry-ground. Using the very convenient and speedy dish test, many different materials ground under various conditions were examined for their toxicity to weevils. These experiments established the following main points:

(*a*) The difference in insecticidal effect between wet- and dry-ground dusts varies considerably with the material ground. It is large for quartz, clinker, zincblende, orthoclase, galena, and magnesite; it is smaller, though definite, for calcite and fluorspar; while it is insignificant for sapphire, rutile and activated gas-mask charcoal.

(*b*) Where it is possible to prepare by precipitation a material of the same chemical composition and crystalline form as a mineral, this is as effective as the wet-ground mineral. This is the case, for example, with zinc sulphide, galena, fluorspar, and silica (Neosyl). On the other hand, with pigment titanium dioxide (Titanox) the product of precipitation is a hydrate which is subsequently dehydrated on heating, and this product is vastly less effective than the ground mineral of the same crystalline form (rutile).

(*c*) The so-called 'micronized' powders, produced by dry-grinding under conditions such that the fine powder is removed from the mill by an air stream as fast as it is formed, are in general as effective as the same powder wet-ground.

(*d*) The nature of the liquid used is immaterial: ether, water, benzene, carbon tetrachloride and medicinal paraffin all give much the same result, except that very viscous liquids like the paraffin slow down the rate of grinding. The duration of grinding, whether wet or dry, does not affect the toxicity of the powder as determined by the dish test once a product containing a substantial proportion of particles of less than 10μ is obtained.

(*e*) When a wet-ground powder is further dry-ground its efficiency in killing weevils is reduced, but the grinding must be carried on for some time before the effectiveness is reduced to that of material initially dry-ground. This is doubtless the case because in the dish test any effective particles present in a powder act on the insects, so that the dry-grinding must be continued until a very large proportion of the particles have been subjected to its deleterious effect. With some precipitated powders dry-grinding reduces the toxicity, while others, for example Neosyl, are unchanged by grinding.

One interesting difference between wet-ground and dry-ground powders is that their particle size distribution differs markedly. For instance, where a powder has been ground both wet and dry to the same median particle size, the wet-ground material is more uniform with particle sizes more narrowly grouped about the average size, whereas the dry-ground dust contains both more very fine particles and more coarse particles than that ground wet. No selection of the relative times of grinding can produce a wet-ground and dry-ground powder of the same particle size distribution. This difference cannot, however, account for the differing toxicity determined by the dish test technique, since the effect of variations in particle size is here eliminated. It is worth noting that such differences alone would be responsible for large differences in weevil mortality in the standard test on wheat.

The difference between wet- and dry-ground powders must therefore lie in the form or structure of individual particles. A likely explanation is that in dry grinding there is a greater opportunity for direct rubbing of particle on particle which may round the corners and polish the faces, producing an amorphous surface akin to the Beilby layer (see, for example, Adam, 1941). It is well known that any unlubricated sliding of one solid on another causes a tearing of the surface. In wet grinding, on the other hand, there is a high probability that a small particle, once formed, will escape further mechanical working by being carried away by the flow of liquid during the approach of the grinding surfaces.

Several lines of evidence give support to such an explanation though they cannot be said to prove it. The Beilby polish layer is generally believed to be a very thin film of a glass-like nature overlying the unchanged crystal lattice. It is in a less stable state than the regular crystal, and should therefore slowly recrystallize ('devitrify') if the temperature is raised to a point somewhat below the melting-point of the solid. Consequently, if dry-ground powders are inferior because they possess a Beilby layer, it should be possible to increase their effectiveness by a suitable heat treatment. Quartz appeared to be the ideal material for this test, since, unlike many of the other dusts which show the dry-grinding effect, it is not liable to undergo any superficial chemical change. A sample of dry-ground quartz was therefore heated in an electric furnace at 1000° C. for 48 hr. This treatment produced no detectable change in particle size or form, but the treated powder proved to be, as expected, very much more effective than the original. This appears to be strong evidence in favour of the theory advanced.

The effect of wet and dry grinding on X-ray diffraction

X-ray powder diffraction patterns would not be expected to reveal a polished layer on the surface of the particles unless it were relatively deep, probably at least several hundred molecules thick. Though powder photographs* of wet- and dry-ground flint and felspar powders showed no difference between the differently prepared samples, it seemed worth while to examine some softer powders in this way since for these the grinding effect would be expected to penetrate very much more deeply than the hard flint and felspar powder and thus perhaps become detectable by X-ray.

Mr H. B. Rooksby of the General Electric Company's Research Laboratories kindly took some X-ray powder patterns of wet- and dry-ground zincblende —differing greatly in their toxicities—and found that the pattern of the dry-ground material was decidedly more diffuse than that given by the same mineral when wet-ground. Work on these lines is being continued and it is hoped that more definite and decisive evidence will be obtained. The electron diffraction technique should be ideal for this purpose since on relatively large surfaces it can detect superficial layers only one or two molecules thick. Unfortunately it appears that no definite technique has yet been evolved for studying powders in the range of 1–10μ diameter.

It follows that in the case of hard materials like flint and felspar the weevil is still the only means of differentiating between wet- and dry-ground dusts.

* Kindly taken for us by Prof. H. L. Riley in the Laboratory of the Northern Coke Research Committee, Newcastle-upon-Tyne.

The effect of surface changes in dusts

Another line of approach to the elucidation of the wet- and dry-grinding difference was to study the toxicity of both wet- and dry-ground powders when the surface of the particle had been modified by chemical etching and other means. A number of different cases have been investigated with the following results:

(1) Dry-ground quartz etched by means of 3% aqueous hydrofluoric acid becomes more effective and indeed equal to wet-ground quartz. Similar etching of wet-ground quartz had no effect on its insecticidal properties. The fact that the difference between wet- and dry-ground quartz is thus eliminated suggests that it is due to some ineffective surface layer on the dry-ground powder which is removed by the acid, thus exposing the effective underlying crystal.

(2) Brief immersion of quartz dusts in water has no effect, but if quartz ground under ether is suspended in a dilute sodium carbonate solution (0·1 *N*) its effectiveness against the weevil is much reduced, and prolonged immersion brings a wet-ground quartz almost down to the level of the dry-ground sample. It seems possible that this effect might be due to a rounding of particles by preferential solution at points and edges (vide rounded glass).

(3) The action of water on felspar dust very markedly increases the effectiveness of the dry-ground material and causes some slight improvement even in the wet-ground dust: X-ray powder photographs taken by Prof. Riley show no difference between water-washed and untreated dry-ground felspar, but this may again be merely because the effect is superficial. A probable explanation is that the action of water on felspar (which is known to result in a preferential extraction of alkali, and to occur very rapidly and to a substantial extent with fine dusts) may leave a superficial layer of hydrated silica which, as the dust is dried at 200° C., would become converted into crystalline quartz.

(4) On suspending anhydrite ($CaSO_4$) in water its effectiveness is considerably increased. The action of hydrofluoric acid on anhydrite also increases its effectiveness a great deal, but this is undoubtedly due to superficial formation of fluorite (CaF_2) which is itself much more effective than anhydrite.

(5) A glass dust which is relatively ineffective becomes much more effective in killing weevils, and comparable with quartz, after a brief etching with 3% aqueous hydrofluoric acid. Any possibility of direct toxic action by traces of soluble fluoride or silicofluoride left by this treatment in any of these experiments is ruled out for two reasons: first, all the powders were thoroughly washed after treatment, and, secondly, direct experiment showed that ground soluble fluoride or silicofluoride (or any other stomach poison) did not kill starving weevils in the 'dish test'.

(6) The effect of heating angular glass dusts up to about 300° C. is to increase slightly their small insecticidal effect, but if the particles are fused into spheres by blowing the dust through a blow-pipe flame and collecting it again from the cooled flame gases, it becomes almost completely harmless to weevils.

While it is not yet possible to give a complete explanation of the foregoing facts, it is clear that they are generally consistent with the idea that the insecticidal effect of a dust is enhanced if the surface of the particles is crystalline or angular in character, while it is diminished if the surface becomes amorphous or rounded.

One point emerging from these observations which should be borne in mind in all future work on dusts is that contact with aqueous liquids may seriously affect their properties so that processes of size classification by sedimentation in aqueous liquids are in many cases inapplicable. In particular, any attempt to study the effect of particle size by comparison of different fractions produced by sedimentation is likely to be invalidated, since these will have been in contact with the fluid for different periods and will thus have been attacked to different extents.

Possible mechanism for the promotion of water loss from insects

It has already been shown that the effect of dusts in promoting water loss from insects is mainly, if not entirely, responsible for their killing action. In the preliminary experiments it was shown that dusts do not act via the respiratory system of the insects; also Germar's (1936) theory that dusts penetrate between the intersegmental membranes and so allow increased diffusion of water to the air seems very improbable both because some dusts can kill although they only adhere in microscopic layers no more than 1 μ thick and also because the surface structure of the particles has so large an influence on their insecticidal effect.

There remains the possibility that dusts increase the transmission of water through the insects' cuticle. Though at first sight this might appear rather unlikely, ample experimental evidence has been obtained that such a phenomenon does indeed occur in certain artificial systems. The capillarity mechanism suggested by Zacher & Kunicke, which supposed that the dusts draw water out of the insects by an action similar to that of blotting-paper, is quite untenable since naturally hydrophobic dusts and also hydrophilic dusts covered with a hydrophobic film can be effective, though water will not rise through such non-wettable powders. It is also difficult to see how the microscopic layer of dust, which in some cases suffices to effect desiccation, would exert such an effect or why, if it did, we should get the observed differences between wet- and dry-ground dusts. Useful light is thrown on the subject by a considera-

tion of what is already known about the structure of insect cuticle.

In the course of the present work it has been found that the permeability of a membrane to water can be enormously reduced by extremely thin wax films, even of molecular dimensions (vide infra). Wigglesworth (1933) and Kuhnelt (1928) have examined the insect cuticle chemically and found that on the outside of the main integument (or *endocuticle*) there is a thin film (the *epicuticle*) which gives none of the characteristic protein, carbohydrate or fat reactions and is only attacked by concentrated caustic alkalis and boiling nitric acid. Subsequently Pryor (1940 *a*, *b*) found that the ootheca of the cockroach, which has the same unreactive character as the epicuticle, is a protein tanned by an orthoquinone, and subsequently impregnated with lipoid. Entomologists have quite generally assumed that it is this epicuticle which is responsible for that retention of water which is essential to the maintenance of the water balance in insects. Apparently this has never been proved on an insect, but Yonge (1932, 1936), examining the cuticle of the lobster which consists of chitin with a superficial layer of lipoid, found that the latter could be partly removed by fat solvents and was then further destroyed or rendered ineffective by some days' immersion in aqueous caustic soda. It then appeared that whereas the untreated cuticle was permeable only to fat solvents, the treated material behaved as a semi-permeable membrane which permitted the free passage of water. It seems possible therefore that the dusts increase the water loss from insects by breaking down the water-resistance of the epicuticle. Though there is no direct evidence as to the nature of this action, it is possible to offer an explanation which, though speculative, is not improbable. It is possible that the epicuticle fat film may be preferentially attracted by the crystalline forces at the surface of a solid particle, and adhere and orientate itself on the crystal rather than on the relatively structureless surface of the cuticle. The continuity of the film might thus be interrupted over submicroscopic areas. The fat film might even spread over the particle by surface migration (since there is much evidence of the mobility of adsorbed layers) and this would certainly account for the high effectiveness of finely powdered activated charcoal which has a large available surface. Such a theory might also account for the remarkable differences between wet-ground and dry-ground dusts. The considerable magnitude of the effect of such minute perforation of the film is intelligible since Brown & Escombe (1900) have shown that the rate of diffusion of a vapour through many small holes in a plate is much greater than through a single hole of the same total area.

In an attempt to evaluate this hypothesis efforts were made to reproduce the effect of dusts by using an artificial membrane-system devised to simulate the insects' cuticle. The results obtained (Alexander *et al.* (1944)) are shortly summarized below.

The effect of dusts on wax-coated membranes

An apparatus was devised to study the rate of diffusion of water through celluloid membranes in contact with water on one side and with dry air on the other. With clean membranes, the rate of diffusion was not at all affected by dusting. When, however, a thin film of beeswax (2–5 mol. layers thick) was applied, the rate of diffusion was reduced to about one-fifth of the original value. When such a wax-coated membrane was dusted with the standard carborundum dust the amount of water diffusing was increased two or three times, and might indeed almost attain the value characteristic of the clean, wax-free, membrane.

Such experiments must, of course, be regarded as a rough analogy, rather than as a true copy of what occurs in the dusting of insects. It is hardly surprising, therefore, that the order of efficiency of a series of dusts is not identical for the insects and the artificial membranes. The general likeness in behaviour between the natural and artificial systems remains nevertheless, and may well be significant.

The fact that the water loss from *dead* weevils is not increased by dusts requires further investigation. It might be explained by supposing that living weevils are constantly renewing some fatty material on the cuticle and that this is responsible for water conservation, but so little is known at the present time about the structure and mode of formation of the epicuticle that any such theories are necessarily wholely speculative. It seems possible that further information might be obtained by a comparative study of the effect of dusts on different types of insects, and some preliminary work of this kind will be reported in Part III. It appears, however, that physiological as well as physico-chemical information is needed for the complete solution of the mode of action of inert dusts.

REFERENCES

Adam, N. K. (1941). *The Physics and Chemistry of Surfaces*, 3rd ed. p. 170.

Alexander, P., Kitchener, J. A. & Briscoe, H. V. A. (1944). The effect of waxes and inorganic powders on the transpiration of water through celluloid membranes. *Trans. Faraday Soc.*, **40**, 10.

Brown, H. T. & Escombe, F. (1900). Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants. *Philos. Trans.* **193**, 278.

Chiu, S. F. (1939). Toxicity studies of the so-called 'inert' materials against the bean weevil, *Acanthoscelides obtectus*. *J. econ. Ent.* **32**, 240.

GERMAR, B. (1936). Versuche zur Bekämpfung des Kornkäfers mit Staubmitteln. *Z. angew. Ent.* **22**, 603.
KUHNELT, W. (1928). Über den Bau den Insektenskelettes. *Zool. Jb.* Abt. 2, **50**, 219.
PARKIN, E. A. (1944). Control of the granary weevil with finely ground mineral dusts. *Ann. appl. Biol.* **31**, 84.
PRYOR, M. G. M. (1940*a*). On the hardening of the ootheca of *Blatta orientalis*. *Proc. roy. Soc.* B, **128**, 378.
—— (1940*b*). On the hardening of the cuticle of insects. *Proc. roy. Soc.* B, **128**, 393.
WIGGLESWORTH, V. B. (1933). The physiology of the cuticle and of ecdysis in *Rhodnius prolixus*. *Quart. J. micr. Sci.* **76**, 269.
YONGE, C. M. (1932). On the nature and permeability of chitin. I. The chitin lining the foregut of decapod crustacea and the function of the tegumental glands. *Proc. roy. Soc.* B, **111**, 298.
—— (1936). On the nature and permeability of chitin. II. The permeability of the uncalcified chitin lining the foregut of *Homarus*. *Proc. roy. Soc.* B, **120**, 15.
ZACHER, F. & KUNICKE, G. (1931). Investigations on the insecticidal action of oxides and carbonates. *Arb. biol. Abt. Anst.—Reichsanst., Berl.*, **18**, 201.

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Inert dust insecticides

Part III. The effect of dusts on stored products pests other than *Calandra granaria*

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(With 1 Text-figure)

The inert dusts used in Parts I and II against the grain weevil have been found effective *in vitro* against a variety of other insects infesting stored products, both larvae and adults. The order, as well as the magnitude, of effectiveness of a series of dusts differs for different species, but the mechanism of action appears to be the same, viz. promotion of evaporation of moisture, leading to death by desiccation. In contrast to the results with *Calandra*, highly adsorbent dusts like charcoal are much more effective on larvae than are mineral powders.

Dusts have a particularly striking action on the mealworm, and this case has therefore been investigated more fully. Dead mealworms when dusted do not lose water nearly as rapidly as live dusted ones.

INTRODUCTION

The desiccation theory of inert dust insecticides is supported by a considerable body of evidence which has been presented in Parts I and II of this series, but there are a number of observations, particularly relating to the loss of weight of dead insects, which are not fully explained by the suggestion that dusts kill insects by impairing the water resistance of a fat layer. It was hoped that further information would be obtained by experiments with insects other than grain weevils. Chiu (1939) studied the effect of dust on the bean weevil (*Acanthoscelides obtectus* Say) and Zacher & Kunicke (1931) made a very extensive, but fragmentary, survey of dust insecticides against many different insects. This evidence was, however, of little value from the standpoint of this investigation since the method of preparation of the dusts was not stated, and, moreover, it was felt as a result of experience gained from the work with weevils, that only strictly comparative experiments were significant. A number of representative dusts were therefore chosen with which to compare by the 'dish test' the resistance of various insects.*

* All the insects were kindly provided by Dr H. H. S. Bovingdon of Messrs Imperial Chemical Industries, Ltd.

MATERIALS

The following dusts were used in the tests:

Sulphur. A sample of ordinary flowers of sulphur was subjected to prolonged dry-grinding in a porcelain laboratory ball-mill. Sulphur was chosen as a typical hydrophobic substance, and one which is practically without effect on *Calandra*.

Talc. Commercial talc powder was used without further grinding since the thin laminae adhere well. Probably owing to its softness, talc is ineffective on weevils.

Quartz (wet-ground). Pure quartz was ground for a long time in a mechanical agate pestle and mortar with benzene.

Quartz (dry-ground). As above but without benzene.

'*Neosyl*'. A proprietary form of precipitated silica.

Carborundum. The same powder ('Grade 800 P.A.H.') as was used in Part II.

Charcoal. Activated gas-mask charcoal was finely ground (dry) in the mechanical mortar.

Alumina. A proprietary brand of calcined alumina.

The tests were made by the simple 'dish test' described in Part II, twenty insects being placed in

a small crystallizing dish with excess dust and stored in a desiccator at 23° C. over calcium chloride and sodium hydroxide.

Toxicity counts after 1, 2 and 3 days gave a rough, but quick and simple, comparison of the killing powers of different dusts without complications arising from differences of particle size. The data are summarized below, those for adult insects being given first.

RESULTS

Stored product pests: adults

(*a*) *Calandra granaria* L. (grain weevil) (data added for direct comparison):

TABLE 1

Dust used	No. dead out of 20 after 1 day	2 days	3 days
Nil (control)	0	0	0
Sulphur	0	0	0
Talc	0	0	1
Neosyl	1	13	20
Carborundum	0	14	20
Alumina	1	17	20
Charcoal	1	15	20

(*b*) *Calandra oryzae* L. (rice weevil):

Behaves very similarly to *Calandra granaria* except that it is more susceptible to all dusts. The two weevils are morphologically very similar.

(*c*) *Tribolium castaneum* Herbst. (red rust flour beetle):

TABLE 2

Dust used	No. dead out of 20 after 1 day	2 days	3 days	4 days
Nil (control)	0	0	0	0
Sulphur	0	0	0	0
Quartz (wet)	1	13	19	20
Quartz (dry)	0	1	3	7
Carborundum	5	16	19	20
Alumina*	19	20	—	—
Charcoal	20	—	—	—

* A brown stain developed round the insect where it lay in the alumina (or Neosyl). It was at first thought that this stain might be connected with the dusting phenomenon. This however was not the case, for when the cause of the stain was investigated it was found to be due to a volatile excretion, which has now been isolated and shown to be impure *p*-ethyl benzoquinone (Alexander & Barton, (1943)).

Against the flour beetle, dusts which are powerful adsorbents (e.g. charcoal and alumina) are very much more effective than hard powders such as carborundum or wet-ground silica. These latter, however, still kill the insects, and a difference between wet- and dry-ground powders still exists. The weevil, on the other hand, is killed equally quickly by both the most effective hard mineral powders and the strong adsorbents. Indeed from results on the weevil alone it appeared likely that there was an upper limit to the effectiveness of dusts since numerous dusts were found to be of the same high order of effectiveness as say carborundum or activated charcoal. Inferior dusts could be brought up to this standard by different treatments (see Part II) but nothing indicated the possibility of going further.

From the results on the flour beetle it appears, however, as if there are two distinct types of dust, the hard mineral powders and the highly surface active powders. This difference is even more apparent with larvae (vide infra).

(*d*) *Tribolium confusum* Duv. (the confused flour beetle):

Results were practically identical with those for *Tribolium castaneum.*

(*e*) *Ptinus tectus* Boield.:

Ptinus tectus (the spider beetle) unfortunately dies very rapidly from starvation and the 'dish test' technique could not be used. The insects were therefore placed on dusted grain and the results obtained showed that they were very much more susceptible to alumina than to carborundum.

(*f*) *Oryzaephilus surinamensis* L. (the saw-toothed grain beetle):

Similar to *Calandra granaria* in its resistance to different dusts, but detailed experiments were not carried out.

(*g*) *Tenebrio molitor* L. (mealworm beetle):

Succumbed very readily to alumina, but owing to a very limited supply of insects no further results could be obtained.

(*h*) *Ephestia kuehniella* Zell. (Mediterranean flour moth):

This appears to be killed equally quickly by alumina and by carborundum. In spite of the fact that the rate of death of controls is very high in dry air (i.e. all dead after 3 days) the death-rate of dusted moths is substantially greater (i.e. all dead after one day).

Stored product pests: larvae

It was not possible to examine the larvae of *Calandra granaria* since these develop inside the grain. The larvae of the flour beetle, the flour moth and mealworm were, however, available and were used in the experiments outlined below.

(*a*) *Tribolium castaneum* larvae:

TABLE 3

Dust used	No. dead out of 10 after 1 day	2 days	4 days	5 days	7 days
Nil (control)	1	1	2	2	4
Nil (control)	1	1	2	3	4
Sulphur	2	3	4	5	5
Carborundum	2	5	6	7	7
Neosyl	5	6	10	—	—
Alumina	10	—	—	—	—

(Carried out at 80 % relative humidity over 20 % NaOH.)

The larvae dusted with alumina were completely shrivelled and hard. The effectiveness of this dust was not at all impaired by use since the sample employed above was re-used for a further experiment with the same result.

(*b*) *Ephestia kuehniella* larvae:

TABLE 4

Dust used	No. dead out of 5 after 1 day	2 days	4 days
Nil (control)	0	0	1
Nil (control)	0	1	1
Sulphur	0	0	2
Carborundum	0	0	1
Neosyl	1	1	5
Alumina	5	—	—

(Carried out at 80% relative humidity over 20% NaOH.)

(*c*) *Tenebrio molitor* larvae (mealworm):

TABLE 5

Dust used	No. dead out of 5 after 1 day	2 days	4 days	6 days	8 days	15 days	36 days
Nil (control)	0	0	0	0	0	0	0
Sulphur	0	0	0	0	0	0	0
Carborundum	0	0	0	1	1	2	2
Neosyl	0	0	1	4	4	4	5
Alumina	1	4	5	—	—	—	—

(Carried out at 80% relative humidity over 20% NaOH.)

TABLE 6

Dust used	No. dead out of 10 after 1 day	3 days	4 days	5 days	7 days	8 days	35 days
Nil (control)	0	0	0	0	0	0	0
Sulphur	0	0	0	0	0	0	0
Carborundum	0	1	1	1	1	2	10
Neosyl	9	10	—	—	—	—	—
Alumina	9	10	—	—	—	—	—

(Carried out at 0% relative humidity over solid NaOH.)

The results show that the effect of hard mineral dusts on larvae is comparatively small, but the adsorbent powders, alumina and Neosyl, are extremely effective.

To determine whether the mechanism of action of dusts was the same as that for weevils some loss-of-weight experiments were carried out with mealworms.

The loss of water from mealworms

The starving mealworm loses both carbon dioxide and water but, as pointed out by Mellanby (1932), the carbon dioxide is produced by oxidation of fats and carbohydrate from food reserves, present in such proportion that a weight of water equal to that of the carbon dioxide is also produced. Consequently the loss of weight of the larvae represents only water loss and this was therefore determined simply by weighing the mealworm in a dish kept in a desiccator over solid calcium chloride or sodium hydroxide at 23° C.

Buxton (1932) and Mellanby (1932) found that the loss of weight of starving mealworms was roughly proportional to the saturation deficiency of the atmosphere except under very unnatural conditions such as high or low temperatures.

Table 7 shows that for mealworms as for weevils the toxicity and the increase in rate of loss of weight produced by dusts run parallel. It is interesting that for the mealworm carborundum is inferior to wet- and dry-ground clinker, but all three hard dusts are much inferior to the adsorbent materials. Table 7 also illustrates the extreme adaptability of undusted mealworms to adverse conditions and the remarkable extent to which they can conserve water. Here mealworms kept in dry air without food for some 34 days progressively reduced their rate of water loss.

Very effective dusts like Neosyl and the alumina induce an enormous increase in the rate of loss of weight, of the order of *forty* times that of the control and in view of this the rapidity of kill of these powders is not surprising. The loss of weight with time of dusted and undusted larvae is shown in Fig. 1. It is worthy of note that the rate of loss of weight of mealworms dusted with alumina is constant up to about 90% of the total water loss. This is not the case for insects dusted with carborundum, as here a definite break occurs in the curve at a time when about half the larvae are dead, and in this respect the mealworms appear to resemble the weevil. It is impossible to kill undusted larvae by desiccating and starving them in dry air since long before any appreciable numbers are dead they pupate and even hatch into adult beetles, and therefore the loss-of-weight curve of undusted larvae cannot be followed throughout as for weevils. The rate of loss of weight of dead mealworms, dusted and undusted, is obviously of great interest, but it was unfortunately not found possible to kill the larvae without their bursting the cuticle of their tail segment. This occurred when they were killed by the most diverse agents (i.e. freezing to −80° C., gassing with hydrocyanic acid, phosgene, chloropicrin, sulphur dioxide or carbon disulphide; or passing an electric discharge through them*). In spite of their open tail ends loss-of-weight experiments were carried out (vide Fig. 1) on dead mealworms. The dead undusted larvae lose water 3–4 times as quickly as the live undusted larvae and the dusted dead mealworms lose water at about 10–15 times as quickly as the live undusted ones. It is not unlikely that the dead undusted larvae lose water quicker because the cuticle has been burst at one point and this may also be responsible for the further increase of rate of water loss on dusting. It is, however, very important to notice that dead

* When the mealworms were killed they excreted a tacky and resinous material which set hard on standing and fixed the dead larvae to the bottom of the dish.

dusted insects do not lose water nearly as rapidly as live dusted insects. It had previously been found (Part I) that live dusted weevils lost water as quickly as both dead dusted and undusted weevils. Thus, in the case of the mealworm it has been clearly demonstrated that the greatest dusting effect occurs only with the living organism. The implications of this result on any theory regarding the mechanism of action of dusts are obviously very great and further experiments with the mealworm are indicated.

While these results do not, as was hoped, provide much further insight into the mode of action of dusts, they do not contradict the tentative ideas advanced in Part II. It is only necessary to postulate that different insects are dependent to different extents on their protective coating of water-resistant material, and perhaps that some are able to renew it more rapidly than others. For instance, the weevil never loses water at more than two or three times its normal rate, perhaps on account of its thick shell, but the mealworm loses enormous quantities of water when dusted. It is quite understandable that highly absorbent powders should have the greatest effect on it.

TABLE 7. *Percentage loss of weight* (% *W*) *and mortality* (% *M*) *of mealworms at* 0% *relative humidity* (10 *insects per test*)

	Time (hr.)													
	18·5		70		96·5		117·5		161·5		189·5		814·5	
Dust used	% *W*	% *M*	% *W*	% *M*	% *W*	% *M*	% *W*	% *M*	% *W*	% *M*	% *W*	% *M*	% *W*	% *M*
Nil (control)	1·4	0	3·5	0	4·7	0	6·4	0	7·4	0	8·8	0	17·1	0
Sulphur	1·1	0	3·9	0	5·4	0	6·3	0	8·2	0	9·6	0	23·6	0
Carborundum	7·5	0	19·0	10	23·0	10	25·7	10	30·0	10	32·8	20	60·5	100
Clinker (wet-ground)	12·4	0	36·7	80	40·7	90	43·6	90	48·1	90	50·4	100	56·8	100
Clinker (dry-ground)	7·0	0	35·2	70	44·9	90	49·1	100	53·8	100	55·3	100	56·4	100
Neosyl	41·7	90	54·5	100	54·6	100	54·6	100	54·7	100	55·0	100	55·0	100
Alumina	39·6	90	54·0	100	54·1	100	54·2	100	54·4	100	54·7	100	54·7	100

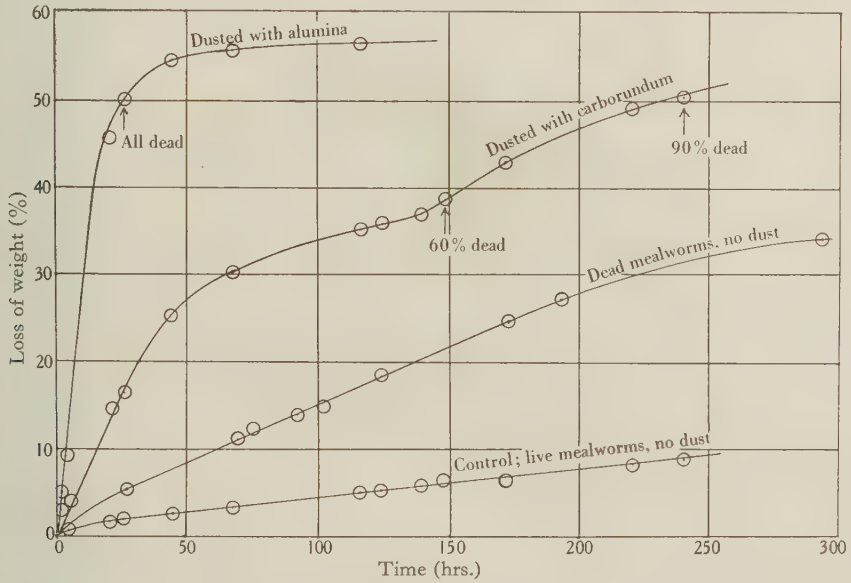

Fig. 1. The effect of carborundum and alumina on the loss of weight of 10 mealworms in still, dry air at 23° C.

REFERENCES

ALEXANDER, P. & BARTON, D. H. R. (1943). The excretion of ethylquinone by the flour beetle. *Biochem. J.* **37**, 463.

BUXTON, P. A. (1932). Terrestrial insects and the humidity of the environment. *Biol. Rev.* **7**, 275.

CHIU, S. F. (1939). Toxicity studies of the so-called 'inert' materials with the bean weevil, *Acanthoscelides obtectus*. *J. econ. Ent.* **32**, 240.

MELLANBY, K. (1932). The effect of atmospheric humidity on the metabolism of the fasting mealworm (*Tenebrio molitor*). *Proc. roy. Soc.* B, **111**, 376.

ZACHER, F. & KUNICKE, G. (1931). Investigations on the insecticidal action of oxides and carbonates. *Arb. biol. Abt. Anst.—Reichsanst., Berl.*, **18**, 201.

(*Received* 5 *November* 1943)

Proceedings of the Association of Applied Biologists

Ordinary meeting of the Association of Applied Biologists held at 11.15 a.m. on Friday, 3 Dec. 1943, at the London School of Hygiene and Tropical Medicine; the President, Prof. W. Brown, F.R.S., in the Chair.

At the morning session: *Discussion on slugs.*

The following papers were read:

I. Introduction and seasonal activity of slugs. By H. F. Barnes, M.A., Ph.D.
II. Field sampling for slugs. By D. C. Thomas, B.Sc., A.R.C.S.

At the afternoon session:

Citrus research in Southern Rhodesia. By W. J. Hall, D.Sc.
History and recent work at the Jodrell Laboratory, Kew. By C. R. Metcalfe, M.A., Ph.D.

Discussion on slugs

I. Introduction. Seasonal activity of slugs

By H. F. Barnes, M.A., Ph.D., *Rothamsted Experimental Station, Harpenden, Herts*

(With 3 Text-figures)

In the spring of 1940 experiments were started by the author, in conjunction with Mr J. W. Weil also of Rothamsted Experimental Station, in order to discover whether substances other than bran could be used satisfactorily as attractants in metaldehyde poison baits for slugs. Accounts of this work have been published (Barnes & Weil, 1940, 1941, 1942).

In 1941 it was found that Miss Barbara Wright (now Mrs Dainton) was investigating the underlying causes of activity in slugs in the Zoological Laboratory at Cambridge, and that Mr D. C. Thomas was experimenting in the South Western Agricultural Advisory Province with methods of sampling for slugs under field conditions. Thereafter a close liaison was maintained by correspondence and meetings. Mrs Dainton carried out experiments on slug activity under laboratory conditions at Cambridge, Mr Thomas made trials under field conditions in Devon in order to determine the best method of estimating slug populations, while the author and Mr Weil at Harpenden restricted their activities largely to investigations on slugs under garden conditions.

It was realized that the lack of exact knowledge concerning the habits of slugs was a source of danger in the interpretation of bait trials, and the investigations at Harpenden quickly developed into an intensive ecological and observational study.

The main method used has been to collect all the slugs seen during visits to various gardens. The unit of time adopted has been 30 min.; an electric torch has been used; the same person has collected the slugs on each occasion; particular care has been taken to include as many as possible of the different ecological sites in the gardens on each visit; and each sample has been sorted into its component species the following morning.

During the past 2 years about fifty gardens have been visited at all hours of the night throughout the year, and sampling has been carried out roughly one night in three on the average. In 1942 twenty-one gardens were visited, 170 ½ hr. samples being taken on 108 different dates, and 30,636 slugs were collected. In 1943, up till the end of October, forty-seven gardens had been visited and 167 ½ hr. samples, producing a total of 35,430 slugs, had been taken also on 108 dates.

One purpose of this paper is to demonstrate that this method of sampling can be trusted to give reliable results and to chronicle the seasonal activity of slugs.

Since slugs are largely nocturnal in habits, all the sampling has to be done after dark, and in addition care has to be taken that the various species are all fully active at the hour when the sampling is carried out. Further, the weather conditions from night to night are rarely exactly the same. Nevertheless, it is possible to obtain almost identical figures of the various species active in a garden either by sampling on successive nights or by sampling twice on the same night (Table 1).

Fig. 1 shows the numbers of *Arion hortensis* obtained by frequently sampling one garden, forty-one

times during 1942 and five times a month in 1943. It will be seen that the numbers were low during the long cold spell early in 1942 and in the drought during Apr.–May, and that there was also a period in June and July when the numbers of individuals active were again low. This was followed by a steady increase in the autumn which reached its maximum during the last 3 months of the year. In 1943 the numbers fell more or less consistently until the lowest were again reached in June and July. Again there was a steady rise in the second half of the year. These general trends are quite obvious in spite of the temporary departures.

Fig. 2 demonstrates that the same trends are shown in the results of sampling other gardens about once a month, whether or not the species of slug is present in large or small numbers in the particular garden.

Monthly averages of the numbers obtained give very similar curves whether they refer to frequent samplings in one garden (Fig. 1), monthly samplings in five different gardens (Fig. 3), or all the samples (170 in 1942 and slightly more in 1943) taken in all the gardens visited (21 in 1942 and 47 in 1943), in some gardens frequently, in others only once or twice.

It is obvious that by taking numerous samples in

TABLE 1. *Collections made after dark on the same night and successive nights in one garden*

Date	Time or duration of collection	*Arion*				*Milax*		*Agriolimax reticulatus*	*Limax maximus*	Total
		ater	*circumscriptus*	*hortensis*	*subfuscus*	*gracilis*	*sowerbyi*			
1941										
10 Nov.	30 min.	0	0	40	9	26	3	53	0	131
11 Nov.	30 min.	1	0	34	25	32	4	37	0	133
9 Dec.	60 min.	3	3	88	22	22	4	68	1	211
10 Dec.	60 min.	5	0	87	33	43	4	47	0	219
1942										
14 Mar.	21.15–21.45	3	0	99	20	35	4	20	1	182
15 Mar.	19.30–20.00	2	2	106	19	28	6	24	0	187
28 Sept.	22.00–22.30	1	0	135	18	82	14	78	1	329
29 Sept.	21.10–21.40	0	1	141	19	97	9	67	0	334
30 Mar.	20.30–21.00	0	0	66	5	193	5	44	0	313
30 Mar.	21.30–22.00	0	0	41	4	142	0	18	0	205
25 Nov.	19·05–19·35	1	0	131	9	80	5	34	0	260
25 Nov.	22.00–22.30	0	1	135	9	89	5	25	0	264
1943										
10 Oct.	21.00–21.30	0	2	137	9	371	17	34	0	570
10 Oct.	22.00–22.30	1	1	142	7	337	5	24	0	517

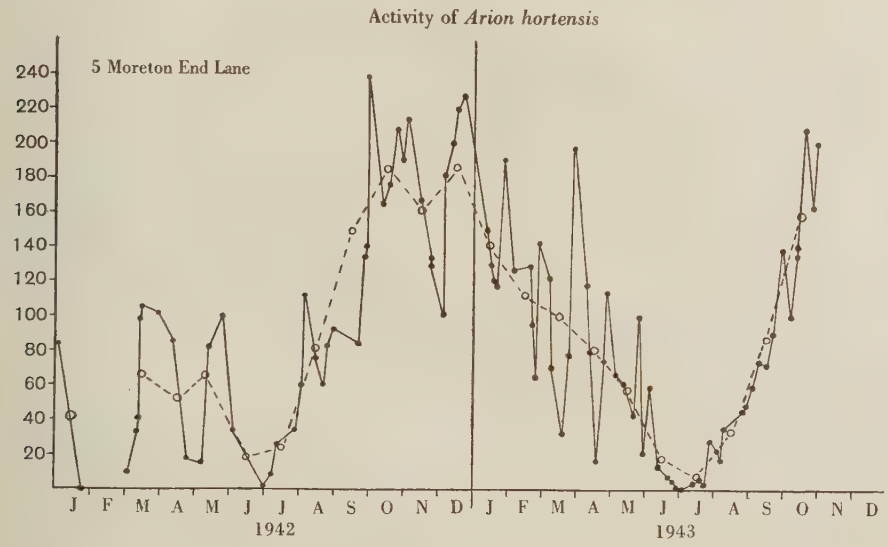

Fig. 1. Actual number per sample (——) and average monthly numbers (-----) of *A. hortensis* in one Harpenden garden which was sampled frequently.

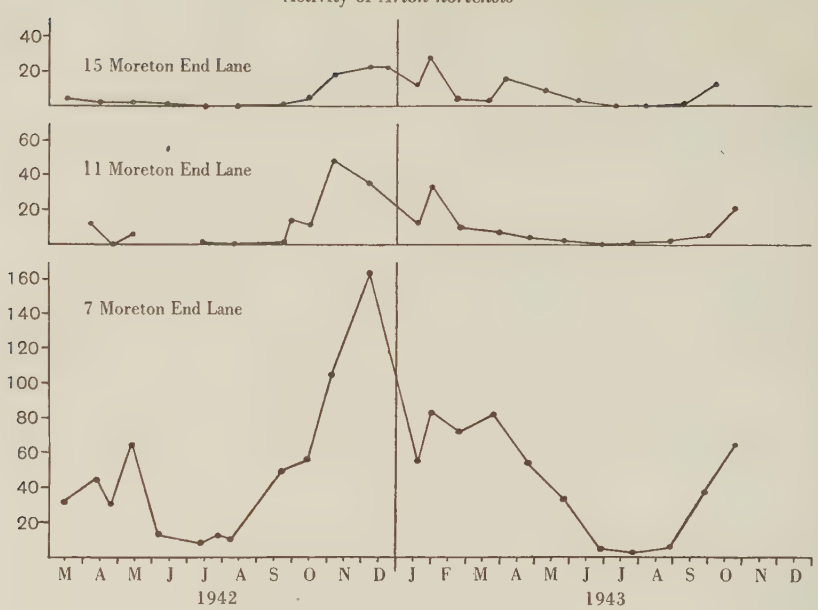
Activity of *Arion hortensis*
15 Moreton End Lane
11 Moreton End Lane
7 Moreton End Lane
M A M J J A S O N D J F M A M J J A S O N D
1942
1943

Fig. 2. Actual numbers per collection of *A. hortensis* in three Harpenden gardens which were sampled about once a month.

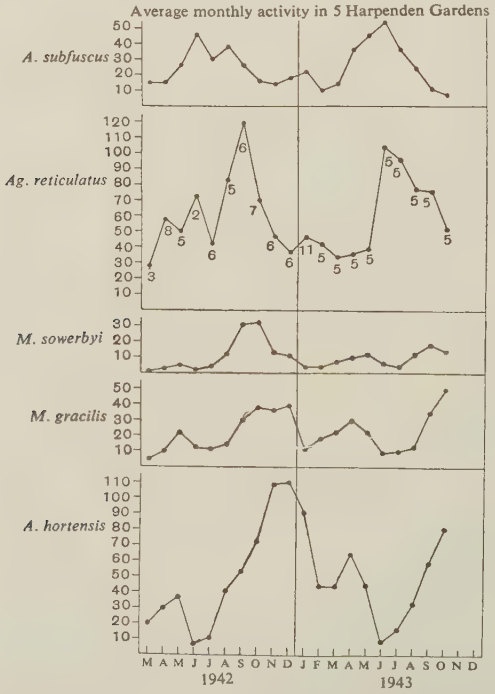
Average monthly activity in 5 Harpenden Gardens
A. subfuscus
Ag. reticulatus
M. sowerbyi
M. gracilis
A. hortensis
M A M J J A S O N D J F M A M J J A S O N D
1942
1943

Fig. 3. The seasonal activity of five species of slugs as shown by the average numbers obtained by $\frac{1}{2}$ hr. collecting after dark in five Harpenden gardens. (The figures under the *A. reticulatus* graph indicate the numbers of collections on which the graphs are based.)

one garden frequently and also by sampling one or several gardens occasionally closely comparable results have been obtained.

Having established the reliability of the sampling method, it is interesting to compare the seasonal activity of the five commonest garden slugs. This is shown in Fig. 3. (The figures on the *Agriolimax reticulatus* curve indicate the numbers of samples on which the averages are based; the same numbers are applicable to the other curves.) *Arion subfuscus* is the first to reach its maximum numbers during what is usually warm and dry weather. *Agriolimax reticulatus* (*agrestis*) comes next, followed quickly by *Milax sowerbyi*. *M. gracilis* and *Arion hortensis* do not reach their peaks until the weather is usually wet and cold.

While these peaks of activity seem to be very definite, it is equally obvious that in different years the actual peaks may be advanced or retarded a few weeks according to the weather conditions. Further, in different parts of the country it is probable that there will be similar differences in the periods of maximum activity.

It is hoped to publish a full account of this and other investigations concerning garden slugs in the near future.

REFERENCES

Barnes, H. F. & Weil, J. W. (1940). Slug control with metaldehyde: experiments on possible substitutes for bran. *Gdnrs' Chron.* **108**, 20.

—— —— (1941). Slug control with metaldehyde: further experiments on possible substitutes for bran. *Gdnrs' Chron.* **110**, 144.

Barnes, H. F. & Weil, J. W. (1942). Baiting slugs using metaldehyde mixed with various substances. *Ann. appl. Biol.* **29**, 56.

II. Field sampling for slugs

By D. C. Thomas, B.Sc., A.R.C.S., *Seale Hayne Agricultural College, Newton Abbot, Devon*

The particular problems arising from any attempt to measure the population of slugs on a field scale have not hitherto received much attention. It therefore appeared desirable to report the results of a study of methods of estimating slug populations.

In the first instance it is necessary to appreciate the type of estimate which can be made and the uses to which the knowledge so obtained can be put. The ideal is a knowledge of the absolute numbers of slugs present per given area in any field. An attempt was made to obtain such a figure by the direct approach of examining soil samples of known size, such as the 4 in. diameter cores used in wireworm population estimates. It was soon apparent that there are considerable errors in this method, the chief difficulties encountered being: squashing of slugs in taking the sample; missing slugs in the examination due to their being covered with particles of soil; the unknown depth to which it is necessary to take the sample to get a true estimate; and lastly, the labour involved by such sampling.

It was therefore obvious that other methods would have to be evolved. The first of these to be tried was suggested by the Northern Group of Advisory Entomologists. It consists of placing a number of 6 in. squares of turf grass side downwards in the field and counting the slugs which have sought refuge beneath them at 10-day intervals. An estimate obtained in this way would include only an unknown proportion of the slugs which have been active during the ten nights over an unknown area of the field and there are certain difficulties and errors in the method. First, an unknown number of slugs may be introduced with the turf, thus in forty 4 in. diam. turves examined, thirty-four slugs were found, equivalent to introducing a population of 425,000/acre. Secondly, there is the high probability of the traps being disturbed by cultural operation during the period necessary to obtain an estimate. Thirdly, it was suspected and has since been demonstrated that inverted turves are not particularly attractive to slugs of the genus *Milax* which are so largely responsible for damage to potato and carrot crops.

It was therefore decided to test the possibilities of using a metaldehyde-bran bait as described by Barnes & Weil (1942). The strength of bait used was 5 teaspoonfuls of bran to ½ teaspoonful of metaldehyde per trap. A series of trials showed that a larger and more consistent catch under various weather conditions could be obtained by covering the baits with 6 in. squares of glass painted black.

By the use of this method observations on the distribution of slugs on arable fields were made. In general it was found that considerable gradients of population might commonly be expected in an arable field and that such gradients did not necessarily coincide with the proximity of the hedgerows. Observations were also made on the relation between climatic conditions and slug activity which confirmed the general impression that warm damp nights without winds induced maximal activity and cold dry windy nights a minimum of activity.

In order to convert the relative figure for population obtained this way into an absolute one it was necessary to know (*a*) the area from which the slugs were attracted and (*b*) the percentage of slugs in that area which were so attracted. Some field experiments were therefore designed to elucidate these points. The technique was to place replicated traps at 10 yd. intervals over a field, encircling alternate traps with a ring of ammonium sulphate, which had been previously found to be an effective barrier to the surface movement of slugs. The diameters of the circular barriers ranged from 3 to 9 ft. The catches on these barriered traps were then compared with the catches on the unbarriered traps on the same night. It was found that in a rough wheat field in winter the maximum distance from which *Agriolimax reticulatus* (Mull.) was attracted was about 3½ ft. and in a field of sparse self-sown oats with a smoother soil surface, about 4½ ft. By continuing to trap within the circles it was possible to arrive at an estimate of 600,000 *A. reticulatus* per acre in a field of wheat very severely damaged by these slugs.

Metaldehyde-bran baits were also found invaluable in investigating slug damage in a field of cow cabbage seedlings at Paignton, south Devon. Replicated traps were placed in the part of the field showing most damage and also in the best part of the field. The catches indicated that while the population of *A. reticulatus* was more or less the same on both areas, that of *M. gagates* was much higher on the damaged part than on the good part. It was concluded that the damage was due to *M. gagates* and not to *A. reticulatus*, a result that could not have been obtained by any mere inspection of the field.

Perhaps the most important use of metaldehyde-bran traps is in connexion with the estimation of the efficiency of slug poisons on a field scale. The technique adopted was to broadcast replicated areas of the field with the poison bait, leaving unbaited areas as controls and, after a suitable interval (usually about 5 nights) to allow all the slugs present to visit the poison, to place the standard metaldehyde-bran baits in the treated and untreated plots. The catches on these traps when examined the following morning give figures for the number of slugs surviving in the different baited areas. By comparing these with the catches on the control areas an index of the killing power of the broadcast poison bait can be obtained.

The poison baits tested in these experiments were Paris Green and bran, 1 lb. of poison to 28 lb. of bran/acre, metaldehyde and bran at the same rate and ½ lb. metaldehyde to 28 lb. of bran/acre. The results show that the percentage kill obtained by Paris Green ranged from 30 to 50 while with metaldehyde kills as high as 60–90% were regularly obtained. Halving the quantity of metaldehyde used, only slightly reduced the kill. The results were similar for all the species of slugs found, with only small specific variation.

It is hoped to publish elsewhere a fuller account of this work and its current developments.

Grateful acknowledgements are due to Dr H. F. Barnes and Mr J. W. Weil of Rothamsted Experimental Station and to Mr L. N. Staniland, Advisory Entomologist, South Western Agricultural Advisory Province, for much useful help and encouragement.

REFERENCE

Barnes, H. F. & Weil, J. W. (1942). Baiting slugs using metaldehyde mixed with various substances. *Ann. appl. Biol.* **29**, 56–68.

Citrus research in Southern Rhodesia

By W. J. Hall, D.Sc., *Imperial Institute of Entomology, South Kensington*

This survey, which covers a period from the beginning of 1927 to the end of 1942, deals with such a wide field that it is only possible to give a very general review.

The climate of Southern Rhodesia is particularly favourable to the cultivation of citrus, especially oranges. Owing to the relatively small European population the local market is limited and citrus plantings on a commercial scale of any size must find their outlet by export to the overseas market.

The British South Africa Company (since 1923 a commercial company) were the pioneers of commercial citrus cultivation in the Colony with three Estates the largest of which is situated at Mazoe, 30 miles north of Salisbury. Southern Rhodesia suffers from the disadvantages that it has no seaboard and is distant from the overseas market. The greater proportion of the crop was railed 1600 miles to Cape Town and thence by ship in cool chamber taking on the average 5 weeks from Estate to consumer. For this reason all problems connected with the carrying qualities of the fruit assumed considerable importance.

The principal variety grown is the Valencia Late Orange tree which produces heavy crops of fruit of excellent quality. It is the practice to raise all trees by bud grafting on Mazoe Rough Lemon which grows wild along the banks of the Mazoe River and is believed to have been introduced by Portuguese traders in the seventeenth century. The Colony has a summer rainy season which lasts about 4 months when an average of 36 in. rain falls, but for the rest of the year there is virtually no rainfall and conse-

quently irrigation is essential for all commercial plantings.

In 1923, 1924 and 1925 severe losses were caused on the Company's Estates by citrus thrips (*Scirtothrips aurantii* Faure) and citrus aphis (*Aphis tavaresi* del G.) and red scale (*Aonidiella aurantii* Mask.) was on the increase. This led to the appointment of an Entomologist early in 1927 and was followed in 1930 and 1931 by the appointments of a Plant Pathologist, a second Entomologist, a Soil Chemist and a Horticulturist. These technical workers were banded together in April 1931 under the title of Mazoe Citrus Experimental Station with headquarters and laboratories on the Mazoe Estate.

The lines of research were dictated by the problems, often immediate, which arose on the Company's Estates and the results were judged by the variation in the actual cost of production per case of fruit sold from year to year.

Owing to the downward trend in the prices realized from 1929 onwards and the fall in production between 1931 and 1935, due to certain problems referred to below, the work of the Station was solely directed to tackling all problems affecting adversely the quantity and quality of fruit produced in an attempt to bring down the cost of production per case by increased tree production of exportable fruit.

Entomological

(1) *Citrus thrips.* This was a major pest and had caused very serious losses prior to 1927. It is particularly serious in Rhodesia because, in addition to the normal ring of scarred tissue round the calyx caused by the insect, a further blemish known as 'tear stain' develops from the original injury during the rains.

Applications of 1% lime sulphur spray with hand-power spray pumps had failed to give control. Studies of the life history and habits of the insect showed that this failure was due to lack of penetration of the spray and lack of understanding when to spray. The purchase of power-spraying machines which would deliver the spray at a constant pressure of 300 lb./sq. in. was recommended. Using the same spray and giving two applications—the first when the fruit was the size of a pea and the second 9 days later—gave satisfactory control at a reasonable cost. Owing to the difficulty of fitting in the double application with the irrigation programme a modification was made later by introducing colloidal sulphur into the spray; this enabled control to be obtained with a single application at a lower cost. The losses from this pest from the 1929 season onwards never exceeded 2% and in all but a very few years were less than 1·5%.

(2) *Citrus aphis.* Although citrus aphis is present to a greater or lesser degree every spring no outbreak of any real importance occurred between 1927 and 1942.

The critical period for aphis is from the time the spring growth first moves until the petals fall towards the end of August. During this period constant observation was maintained for local colonies and these were at once sprayed with nicotine and soap or lime sulphur and nicotine. Once the petals have fallen the danger is much less but groves in which aphis was present were cleaned up by the introduction of nicotine in the thrips spray which was applied about the end of September.

(3) *Red scale.* This pest was not particularly serious in 1927 but it was on the increase and fumigation was already being practised. This was extended to an annual fumigation of all trees in view of the necessity for all fruit for export being entirely scale free. Pack House losses at Mazoe from the fruit from a representative block of 15,000 trees over a period of 10 years averaged under 1%.

Whilst reasonable control on bearing trees was secured, young non-bearing trees gave much trouble. The life history of the insect under local conditions was worked out and the most favourable time to apply control measures determined. Satisfactory results with sprays were not obtained and box fumigation was resorted to despite the practical difficulties and disadvantages of this method.

(4) *Cotton boll worm* (Heliothis armigera *Hubn.*). An outbreak of this pest occurs every year, the moths being attracted by the blossom and the subsequent caterpillars feeding on the newly set fruit. Fortunately there was only one serious outbreak between 1927 and 1942, although losses were caused to a greater or lesser degree in a few groves in most years. The life history and habits of the insect were fully studied, but no satisfactory control measures resulted. The Tachinid and egg parasites were also studied; of the latter *Telenomus ullyetti* Nixon showed possibilities and was bred in the insectary during the winters of 1938 and 1939, but the subsequent liberations in the field gave inconclusive results owing to the extremely mild outbreaks of boll worm in those years.

Plant pathology

Plant diseases are fortunately not so prevalent in Southern Rhodesia as they are in many citrus-growing countries. The only serious tree disease is scaly bark of which a very virulent strain occurs. The few trees that develop this complaint annually are eradicated.

Fruit on the tree is seldom affected by fungi unless it possesses some mechanical or physiological injury.

Wastage in the Pack Houses and in transit is due to green mould; blue mould is comparatively rare. Wastage in all its aspects was carefully studied and care in handling of the fruit in all its stages found to be the most important factor in minimizing losses from this cause. The incidence and development of wastage under varying conditions of temperature as

well as the prevention of mould decay in transit and storage by treated wrappers and other devices was also investigated. Other lines of research were loss in weight of the fruit and its relation to wastage, physiological injuries such as pit marking and low temperature injuries and the breakdown of fruit due to such injuries, the types of decay found in stored oranges and the subject of latent infection.

A considerable amount of work was done on the maturity tests of the fruit in relation to the standards laid down by the South African Citrus Exchange for all fruit exported through a South African port.

Experimental work on the so-called artificial coloration of oranges was carried out and was introduced as part of the routine in the Company's Pack Houses. The replacement of the Washington Navel variety by Valencia Late trees subsequently made it no longer necessary to continue this process.

The increase in recent years of sooty blotch and flyspeck on the fruits was investigated and means of removing these blemishes were devised by passing the fruit in the Pack Houses through baths containing a bleaching medium.

Soil problems

A complete soil survey of all the Company's Estates was undertaken and differential treatment was based on the results obtained. A study was made of the nature of fertilizers required by the citrus tree, and the best method and time of application. Experiments were carried out over a period of 4 years to determine the effects of differential fertilizer treatments on crop production and fruit quality. Preparation of compost was organized as a routine task on all Estates; it was made from two sources: (*a*) available organic matter such as Sunn hemp, wheat straw, maize stalks, river reeds, grass and the like, (*b*) the waste products from the Citrus By Products Factory at Mazoe.

Irrigation and cultural methods were considerably modified as a result of investigations carried out and the practice of cover cropping between the trees bandoned in favour of clean cultivation.

In 1933 large blocks of trees suddenly showed signs of distress and became unthrifty. These trees were situated in areas where storm water and prolonged irrigation by the furrow method had given rise to erosion or silting. A big programme of soil reclamation on the one hand and removal on the other was undertaken and the trees showed an immediate response out of all proportion to the work done. Shortly afterwards large blocks of trees where no erosion or silting had taken place also deteriorated in similar fashion. It was found that there was a relatively sharply defined low limit for the available nitrogen reserves in the soil required by the tree and that despite the annual dressings of fertilizer and manure these reserves had been gradually depleted until a critical low level had been reached. Soil reclamation and increased nitrogen dressings throughout had the most striking results in bringing the sickly trees back to full health and vigour.

The production of fruit known locally as 'hard fruit' developed from a rarity in 1930 to a major problem in a very few years. Trees carrying such fruit became entirely unthrifty and changed in character and any fruit carried was hard to the touch, contained gum pockets in the albedo tissue and juice sacs, and had a much lowered juice content. This was found to be due to the gradual depletion of the boron content of the soil. The application of from 2 to 8 oz. of borax per tree according to size and condition brought the trees back to full health and vigour in 18 months.

Conclusion

The task facing the Station on its establishment in 1931 was indicated earlier. Production reached its lowest ebb during the 1935 season, but after that it steadily improved until by 1939 tree production was higher and cost of production lower than it had ever been before. This high rate of production was maintained until 1942, but owing to the War the cost of production figures were no longer comparable.

For the time being the Station has been closed down, but two of the staff still remain and there is every reason to believe that it will be revived once the War is over.

Citrus growing, as is the case with other fruit and agricultural crops, is a highly scientific and technical business. It is hoped that, in the reorganization that must take place after the War, the importance of research will be accorded a far greater measure of recognition than in the past, not only in the agricultural policies of the Governments within the Empire but also by growers both large and small.

History and recent work of the Jodrell Laboratory, Kew

By C. R. Metcalfe, M.A., Ph.D., *Royal Botanic Gardens, Kew*

There were no facilities for research in plant anatomy and physiology at the Royal Botanic Gardens, Kew until 1876, when Mr T. J. Phillips Jodrell founded and equipped a laboratory there at a cost of £1500. The original building has been very little altered except that an artist's studio and improved facilities for photography were added in 1934. At the time of its foundation the laboratory was as well equipped for research purposes as any of those on the Continent where British plant anatomists and physiologists had

previously been forced to work, owing to the lack of adequate facilities and instructors in this country. Since its foundation, nearly 400 papers have been published concerning the investigations carried out at the laboratory, including a number of important contributions to the Philosophical Transactions of the Royal Society. The period of most intense activity was when Dr D. H. Scott occupied the position of Honorary Keeper (1892–1906), since his genial personality and wide knowledge attracted many visitors who subsequently became well-known botanists. In particular Scott's own researches on palaeobotany and those of Brown and Escombe on transpiration, both of which were completed during this period, will always stand out as landmarks in the development of British botany.

Quite early in its history the laboratory became a centre for the study of anatomy in relation to taxonomy, the practical application of which was the identification of vegetative material such as herbarium specimens devoid of flowers or fruits, timbers, fibres and other miscellaneous material. This line of work has been carried on ever since, and in recent years the anatomical method has proved useful in many curious ways, such as detection of the admixture or substitution of other plant hairs in Kapok (*Ceiba pentandra*) intended for use in life-saving apparatus, the identification of fibres in old ropes in order to determine whether they would be suitable for certain commercial purposes, while, from time to time, assistance has been given in connexion with criminal investigations. My predecessor, Mr L. A. Boodle, was the chief exponent of systematic anatomy at Kew for nearly 30 years, and it is to be regretted that he published very little concerning this subject of which he was a master. An attempt to remedy this defect is now being made, however, by writing a new book on the systematic anatomy of the dicotyledons. This is being done with the collaboration of Dr L. Chalk, of the Imperial Forestry Institute at Oxford, and Dr C. L. Hare, who is working at the Jodrell Laboratory. The new book will include the essence of Solereder's classical work on the same subject, the English edition of which was partly translated from the German text by Boodle and published in 1908. In addition it will include a summary of the literature which had appeared since 1908, as well as many unpublished observations based on the extensive slide collections at the Jodrell Laboratory and the Imperial Forestry Institute.

Visitors have always been welcome at the laboratory either for short or long periods. One consequence of wartime conditions is that three of those who are working there at the present time are Continental botanists who have taken refuge at Kew. They are Dr L. Portheim, a physiologist from Vienna, Dr M. Skalinska, a cytologist from Poland, and Dr E. Glaznerova, a biochemist and physiologist from Czechoslovakia.

Since the war began a certain number of investigations which are rather outside the usual scope of the laboratory have been undertaken. For instance the fibre of the common stinging nettle (*Urtica dioica*) has been the subject of an extensive reinvestigation in collaboration with other institutions and firms. This, besides demonstrating the very great tensile strength of the fibre, has also led to the preparation on a laboratory scale of special kinds of paper, textile yarns, and artificial silk from the nettle stems. Technical assistance has also been given which has led to the establishment of a factory where pith, pegwood, charcoal and other miscellaneous items, which were previously imported from the Continent, are being prepared from indigenous plant material which has hitherto been regarded as useless. Determinations of the rubber content of a number of laticiferous members of the Compositae and Euphorbiaceae which can be grown in a temperate climate have also been undertaken. The rubber estimations have been done almost entirely by Dr E. Glaznerova, who has confirmed that most of the investigated plants contain very little rubber, or rubber which is admixed with too large a proportion of resin to make it suitable for commercial use. On the other hand, the dried roots of the Russian plant known as Kok-saghyz (*Taraxacum kok-saghyz*) have been found to contain an average of 9% of rubber when one year old. Sample sheets of rubber from this plant have been prepared by a combination of mechanical and chemical or retting processes by Mr F. Richardson, the laboratory assistant. That the chemical and physical properties of the rubber are not much inferior to those of *Hevea* rubber has since been proved by Mr G. Martin and his colleagues on the London Advisory Committee for Rubber Research. One-year-old roots of Krim-saghyz (*Taraxacum megalorrhizon*) contain a smaller percentage of rubber, the qualities of which have not yet been fully investigated. This work has confirmed the Russian claim that good quality natural rubber can be produced in a temperate climate. In Great Britain, however, there is not at present sufficient land or cheap labour to enable the rubber to be produced in commercial quantities. It is, nevertheless, rather difficult to predict if, or to what extent, Kok-saghyz rubber will assume economic importance elsewhere, especially after sufficient time has elapsed for a high-yielding strain to be produced, and for methods of cultivation and extraction to be improved.

Obituary Notices

Sir EDWIN JOHN BUTLER, C.M.G., C.I.E., F.R.S.

President of the Association of Applied Biologists, 1928–9

Edwin John Butler, the son of Thomas Butler, Resident Magistrate, was born in 1874 in County Clare, Ireland, and educated first at Gainsborough in Lincolnshire, later, owing to illness, under a tutor, and then at Cork, ultimately entering Queen's College, where in 1898 he graduated in medicine with honours. He was never attracted to medicine, however, and, like other great men, forsook the profession for which he was trained to take up the study in which he delighted, in his case the fungi. Marcus Hartog was responsible for arousing his enthusiasm for the aquatic Phycomycetes. In 1899 he was awarded a travelling scholarship by the Commissioners of the 1851 Exhibition and spent two years studying under Cornu and van Tieghem in Paris, under Poirault at Antibes, under Oltmanns at Freiburg and then at the Jodrell Laboratory, Kew, where D. H. Scott was Keeper.

In 1900 Butler was appointed Cryptogamic Botanist to the Government of India and commenced his duties in Calcutta the following year. In 1902 he was transferred to Dehra Dun, and in 1905 went to the Agricultural Research Institute, Pusa as Imperial Mycologist. In 1919 he became joint Director and in 1920 Agricultural Adviser to the Government of India. In July 1920 he left India to become Director of the Imperial Bureau of Mycology, which he established on a solid basis. In 1935 he resigned the Directorship to become Secretary of the Agricultural Research Council, an appointment which he retained until, in January 1941, ill-health compelled his retirement. Following an attack of influenza he died on 4 April 1943.

Many honours came to him. In 1921 he was appointed C.I.E., in 1926 was elected F.R.S., in 1932 became a C.M.G., in 1938 received the LL.D. *honoris causa* of the University of Aberdeen, and in 1939 was knighted. Among many honours from scientific societies he was President of the Association of Applied Biologists in 1928–9.

Within the limits of this short notice it is impossible to indicate the scope of his contributions to mycology and plant pathology. Among his outstanding contributions were his classical paper on the genus *Pythium*, his study on the bud-rot of the coconut palm leading directly to the control of that dread disease, and his book on *Fungi and Disease in Plants*, but perhaps more important even than these was the influence he exercised on the development of mycology and plant pathology first in India and later throughout the Empire. No man contributed more to this advancement than Butler and this was done in so unobtrusive a fashion that only those intimately associated with him could rightly appraise the immense industry, untiring zeal, and tremendous mental energy he put into everything he undertook. He is survived by Lady Butler, a son, and two daughters.

S. P. WILTSHIRE

Sir EDWARD B. POULTON, M.A., D.Sc., LL.D., F.R.S.

President of the Association of Applied Biologists, 1922–3

In the death of Sir Edward Bagnall Poulton biology has lost one of its greatest disciples of the Darwinian school. Born on 27 Jan. 1856, he was educated at Jesus College, Oxford, and graduated with a First Class in the Honours School of Natural Science. His early scientific life was spent in zoological teaching in Oxford and among his first researches was his discovery, in 1888, of vestigial teeth in the *Ornithorhynchus*. His dominating interest, however, was in insect life and during 1884–8 he published a series of papers on the colours of Lepidopterous larvae and pupae in relation to their surroundings. This work was the result of many observations and experiments made to evaluate the significance of such colours in nature. In 1890 his book *The Colours of Animals* was published and 3 years later he succeeded J. O. Westwood as Hope Professor of Zoology at Oxford—a post which he held until 1933. From about this time the applications of Darwinism to the theory of mimicry became one of Poulton's most enduring interests. He devoted a great amount of time and energy to massing data on the subject, particularly in relation to butterflies. In this connexion he established a wide circle of collaborators and correspondents and constantly urged naturalists to observe and experiment in the field. It was mainly through Poulton's efforts that considerable light has been thrown on such polymorphic species as *Papilio dardanus* and others. He maintained that natural selection explains the phenomena of mimicry better than any other theory. The selective agents he claimed were birds and other vertebrates along with insect

predators. Critics often assailed him over his contentions, but to this day no alternative explanation has yet been offered that fits the facts as adequately as the selection theory.

Poulton devoted much time to the care of the extensive collections of insects in the Hope Department which he brought to the status of being among the most important in the world. He received many distinctions and was Honorary Doctor of six universities. He was three times President of the Entomological Society of London (1903–4, 1925–6, 1933) and a Vice-President for five separate periods and was, at the time of his death, Honorary Life President of that Society. In 1922–3 he was President of the Association of Applied Biologists and his address, which was on the relations between pure and applied biology, was published in the *Annals of Applied Biology* (1924, II, 271). Personally Poulton helped and inspired many a young naturalist besides being a good friend to many of the older ones. Being a great believer in personal contacts he was a most constant attendant at the meetings of various associations and societies. His death took place on 20 Nov. 1943. A. D. IMMS

Lt.-Col. Sir DAVID PRAIN, C.M.G., C.I.E., V.M.H., M.A., M.B., LL.D., F.R.S.

President of the Association of Applied Biologists, 1919–21

David Prain was born on 11 July 1857, at Fettercairn, Kincardineshire and died on 16 March 1944, at the Well Farm, Warlingham, Surrey. Educated at his parish school, the Aberdeen Grammar School, and the Universities of Aberdeen and Edinburgh, he was thoroughly grounded in the humanities, in science, and in medicine. In 1882 he was Demonstrator in Anatomy at the Edinburgh College of Surgeons and in 1883 at Aberdeen University. In 1884 he entered the Indian Medical Service and spent 2 years attached to native regiments in various parts of India.

In 1887 Prain became Curator of the Herbarium and Library of the Royal Botanic Garden, Calcutta and, 11 years later, was appointed Superintendent of the Garden and Director of the Botanical Survey of India. From 1895 to 1905 he was also Professor of Botany in the Calcutta Medical College; from 1898 to 1907 Trustee of the Indian Museum; and in 1903–4 Secretary of the Board of Scientific Advice for India. As Curator Prain became keenly interested in economic botany, and among his activities was the successful development of the Indian cinchona industry. He travelled widely in India, especially in the N.W. Frontier and Himalayan regions, and, in 1902, accompanied the Younghusband mission to Lhasa. He was a fecund worker in systematic botany, and his papers are models of scientific writing: in 1903 he published a *Flora of Bengal*.

In 1905 Prain returned to this country as Director of the Royal Botanic Gardens, Kew, and, for 17 years, splendidly maintained the great Kew tradition. In spite of the hampering influences of the 1914–18 war he initiated major developments in all the Kew departments and extended the almost innumerable activities with which Kew is identified. In 1915 the first Plant Pathological Laboratory of the Ministry of Agriculture was founded as a Kew department and, in 1921, Prain was largely responsible for the establishment at Kew of the Imperial Bureau of Mycology. Prain's services were on call by Government Departments and many scientific bodies, but he found time to carry out original research, mainly on the African flora, and to edit the *Botanical Magazine*.

On his retirement from Kew in 1922 Prain, at the age of 65, became Director of Forest Products Research in the D.S.I.R., carried out investigations in this field as well as continuing his researches in systematic botany, and as Trustee or Member of the Governing bodies of Research Institutes and as Officer of all the leading societies he exercised during 20 years a profound influence on the development of botanical and horticultural science in this country. He was President of the *Association of Applied Biologists* from 1919 to 1921, and his foresight and wise counsel led the Association safely through these critical years and laid the foundations for its later progress.

Sir David Prain was a great scientist and a great administrator. His mind was a rich store of scholarly learning and practical worldly experience, and, although he did not suffer fools gladly, he gave without stint to all who asked his help. Especially was he generous to young scientific workers, many of whom owe to him more than can easily be put into words. Sir David Prain was a great man, wise and kind, and his memory will long be an inspiration to all who knew him. W. B. BRIERLEY

Report of the Council of the Association of Applied Biologists for the year 1943

The Officers and Council of the Association were as follows:

President: Prof. W. Brown, M.A., D.Sc., F.R.S.

Vice-Presidents: E. W. Mason, M.A., H. W. Miles, D.Sc.

Hon. Treasurer: W. C. Moore, M.A.

Hon. Secretaries: General and Botanical: W. P. K. Findlay, D.Sc.; Zoological: H. F. Barnes, M.A., Ph.D.

Hon. Editors of Annals of Applied Biology: General and Botanical: Prof. W. B. Brierley, D.Sc.; Zoological: C. T. Gimingham, B.Sc., F.I.C.

Council: *F. C. Bawden, M.A., D. E. Green, M.Sc., S. E. Jacobs, Ph.D., *E. W. Mason, M.A., *H. W. Miles, D.Sc., E. A. Parkin, Ph.D., C. Potter, D.Sc., *H. Shaw, Ph.D., W. Steer, B.A., I. Thomas, Ph.D., W. A. R. Dillon Weston, M.A., Ph.D., *Miss A. P. Wilson, A.R.C.S.

The Association has again enjoyed a successful year of progress. Six meetings have been held in London, at which the attendance was very satisfactory, averaging 46 members and 22 visitors at each meeting. The total membership now stands at 344, which shows a net increase of 16 over the previous year. Twenty-five new members were elected and only 7 resigned. The Council has to record with great regret the deaths of Sir Edwin Butler and Prof. Sir Edward Poulton. Both were Past-Presidents of the Association and had taken an active part in its development. Prof. Poulton, who joined the Association in 1907, was one of the oldest members of the Association and held the position of Vice-President from 1909 to 1913.

The following papers were presented to the Association during the year:

12 Feb. Presidential address, by Dr H. Martin: *War problems and post-war responsibilities of the Association.*

26 Mar. Address by Prof. J. Bayley Butler: *Some aspects of applied biology in Eire*, including notes on the following subjects: (*a*) new methods for examination and treatment of dry rot; (*b*) some waterworks problems; (*c*) an attempt to naturalize Irish freshwater shrimps at Balmoral; (*d*) bird pests of structural timber and steel; (*e*) ants, cockroaches and golden spider beetles in institutions.

This was followed in the afternoon by a general discussion on: *The future aims and policy of the Association.*

11 June. *Discussion on composts, their preparation and value.*

Morning session: E. M. Crowther: 'General introduction'. F. A. Secrett: 'The use of composts in market-gardening'.

Afternoon session: M. C. Rayner: 'The use and significance of composts in forestry'. W. F. Bewley: 'The use of straw in glasshouses'.

1 Oct. *Work on control of insects which attack stored foodstuffs.*

Morning session: G. V. B. Herford: 'Survey of the work of the Pest Infestation Laboratory'. T. A. Oxley and R. W. Howe: 'Factors influencing the course of an insect infestation in bulk wheat'. M. E. Solomon: 'The behaviour of mite populations in stored grain and flour'.

Afternoon session: W. Burns Brown: 'The role of the chemist in research on fumigation'. E. A. Parkin: 'Control of the granary weevil with finely ground mineral dusts'.

23 Oct. Joint meeting with the British Mycological Society.

Morning session: W. C. Moore: 'Introduction'. D. E. Green: 'Onions—yellow strip and neck rot'. R. W. Marsh: 'Notes on summer spraying of onions'. G. H. Brenchley: 'Club root'.

Afternoon session: W. Brown: 'Some problems relating to the cultivation of winter lettuce'. C. J. Hickman: 'Some observations on the sources of infection of *Didymella Lycopersici*'. D. E. Green: 'Spraying against tomato blight'.

3 Dec. *Discussion on slugs.*

Morning session: H. F. Barnes: 'Introductory and seasonal activity'. D. C. Thomas: 'Field sampling'.

Afternoon session: W. J. Hall: 'Citrus research in Southern Rhodesia'. C. R. Metcalfe: 'Investigations in progress at the Jodrell Laboratory, Kew'.

The Council wishes to take this opportunity for expressing its sincere thanks to the Authorities of the London School of Hygiene and Tropical Medicine and of the Imperial College of Science and

* New members for 1943.

Technology for so kindly affording the Association accommodation for its meetings.

The Council has carefully considered suggestions put forward by Members at the meeting on 26 Mar. regarding the future organization and developments of the Association and has decided in the light of these to modify the rule regarding the method of electing new members of Council. This change in the Rules will of course require to be submitted to an Extraordinary General Meeting of the Association.

As a result of suggestions made by a group of Members the Council considered proposals for setting up a 'Plant Pests and Diseases Committee' to advise the Council as to what action should be taken in relation to certain urgent problems in this field. It has been decided that such a Committee shall be formed, and it is hoped that it will shortly be able to get to work.

It may interest Members to know that the Biology War Committee which was set up in 1941 at the request of the Association and two other biological societies has continued to function actively during the past year. Representatives of the Association were present at each of the meetings held.

The *Annals of Applied Biology* was issued in a new economy format with double columns and a smaller fount. Vol. **30** (1943) comprised pp. viii + 400 and 9 Plates and a Frontispiece as against pp. ix + 439 and 15 Plates for vol. **29**. Including papers published in 'Proceedings', vol. **30** contained 69 papers, 57 being by Members of the Association. The subjects may be classified roughly as follows: general applied botany, 12; mycology and fungus diseases, 9; viruses and virus diseases, 13; entomology and insect pests, 16; plant protection, insecticides and fungicides, 10; helminthology and nematode diseases, 5; applied microbiology, 2; general, technique and statistics, 2.

Publication of the *Annals* was unfortunately delayed owing to war-time production difficulties. The several numbers of vol. **30** were issued on the following dates: No. 1, 19 May 1943; No. 2, 13 August 1943; No. 3, 9 December 1943; No. 4, 11 January 1944.

In the last Report of the Council (vol. **30**, 198) in the list of speakers at the Symposium on 'Domestic entomology' the name of the first speaker was inadvertently given as P. A. Buxton. It should have been F. H. Braybrook: 'Shell Film Unit's film on Malaria'.

Report of the Honorary Treasurer for the year ended 31 December 1943

During the year ended 31 December 1943, the amount received for subscriptions and entrance fees, including arrears, was £402. 14*s*. 6*d*., which was £26. 10*s*. 0*d*. more than in 1942. Subscriptions not paid by the end of the year, but considered good, amounted to about £20.

Income from the sale of the current volume of the *Annals of Applied Biology* amounted to £672. 13*s*. 4*d*., an increase of £179. 6*s*. 6*d*., due partly to a greater demand for the *Annals* but mainly because the Council felt obliged to raise the price of the Journal as from 1 January from 40*s*. to 50*s*. per volume. The volume contained 400 pages compared with 439 pages in 1942, but the further change of format that was made resulted in an appreciable increase in the costs of producing it, for these costs rose by £244. 9*s*. 1*d*. to £1194. 5*s*. 7*d*. The improved sales of back volumes and parts was again very marked and realized £175. 8*s*. 3*d*. compared with £82 in 1942 and £14 in 1941. The balance against the *Annals* on the year's working was £397. 17*s*. 0*d*.

Expenditure for the year exceeded income by £22. 2*s*. 10*d*., and after all obligations had been met, the assets of the Association at the end of the year amounted to £1128. 1*s*. 11*d*., of which £715. 13*s*. 4*d*. was represented by National Savings Certificates, £404. 13*s*. 9*d*. by investment in a newly opened Post Office Savings Bank Account, and £115. 11*s*. 0*d*. by the estimated value of the stocks of the *Annals*. The total also included a sum of £150 allocated to the Association by the Council of the Royal Society from the special Rockefeller Foundation Grant in aid of Scientific Publications, a grant which the Council wish to acknowledge with gratitude.

W. C. MOORE
Hon. Treasurer

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1943 *Cr.*

Expenditure	£	s.	d.	Income	£	s.	d.
To Estimated Value of Stock, 1 January 1943	195	3	0	By Sales—Current Volume	672	13	4
To Cambridge University Press	1194	5	7	By Sales—Back Volumes, Parts and Sets	175	8	3
				By Sales of Reprints and Adverts	27	19	0
				By Estimated Value of Stock, 31 December 1943	115	11	0
				By Balance, carried down	397	17	0
	£1389	8	7		£1389	8	7

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1943 *Cr.*

Expenditure	£	s.	d.	Income	£	s.	d.	£	s.	d.
To *Annals of Applied Biology*, balance brought down	397	17	0	By Members' Subscriptions:						
To Printing and Stationery	4	9	11	Arrears	15	0	0			
To Postages and Cheque Stamps	12	18	11	Entrance Fees	15	4	6			
To Subscription—Parliamentary Science Committee	10	10	0	Current	372	10	0			
To Sundry Out-of-Pocket Expenses of Editors, Secretaries and Treasurer	20	0	6					402	14	6
To Audit Fee Reserve	5	5	0	By Interest Receivable:						
				National Savings Certificates	20	17	1			
				Post Office Savings Bank Account	4	13	9			
				Bank Deposit Account		13	2			
								26	4	0
				By Balance, being Excess of Expenditure over Income for the Year				22	2	10
	£451	1	4					£451	1	4

BALANCE SHEET, 31 DECEMBER 1943

Liabilities and Surplus	£	s.	d.	£	s.	d.	Assets	£	s.	d.	£	s.	d.
Sundry Creditors:							Cash at Bank:						
Cambridge University Press	348	5	0				Current Account	283	12	7			
Audit Fee Reserve	5	5	0				Deposit Account	26	0	0			
Sundry Expenses	16	6	3				Post Office Savings Bank Account	404	13	9			
				369	16	3					714	6	4
Subscriptions and Entrance Fees, paid in advance				22	12	6	500 National Savings Certificates				715	13	4
Life Membership Fund Reserve				25	0	0	Stock of *Annals of Applied Biology* at estimated value				115	11	0
Excess of Assets over Liabilities:													
As per Balance Sheet of 31 December 1942	1000	4	9										
Add: Rockefeller Foundation Grant (per Royal Society)	150	0	0										
	1150	4	9										
Less: Balance of Income and Expenditure Account for 1943	22	2	10										
				1128	1	11							
				£1545	10	8					£1545	10	8

W. C. MOORE, *Hon. Treasurer*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association according to such books, vouchers and documents.

(Signed)
H. J. COX & CO.
Incorporated Accountants
Auditors

HARPENDEN, 12 *February* 1944